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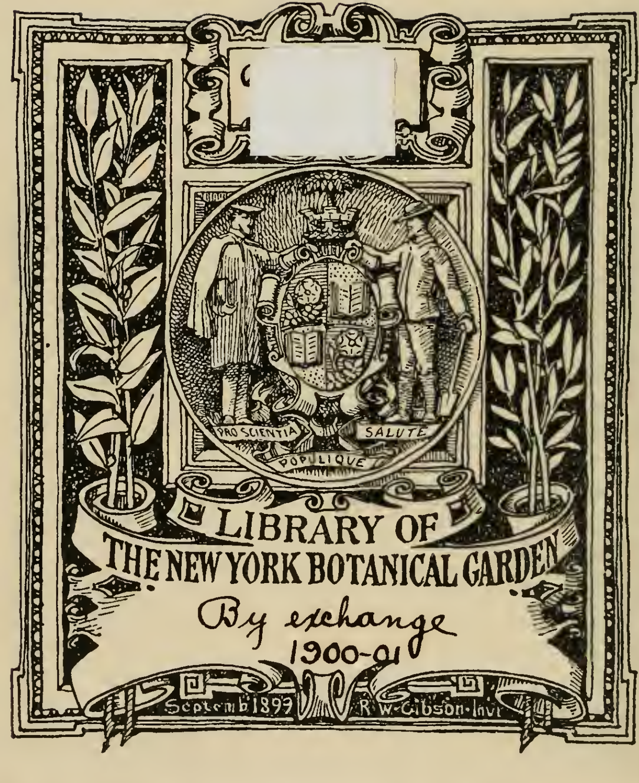
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BULLETIN No. 23. V. P. P. 76.

U. S. DEPARTMENT OF AGRICULTURE,
DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY.
B. T. GALLOWAY, Chief.

SPOT DISEASE OF THE VIOLET.

(*Alternaria violæ* n. sp.)

BY

P. H. DORSETT,
Associate, Division of Vegetable Physiology and Pathology.

Issued November 28, 1900.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1900.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Division of Vegetable Physiology and Pathology,
Washington, D. C., August 29, 1900.

Sir: I respectfully transmit herewith a paper by Mr. P. H. Dorsett, of this Division, giving the results of some investigations of a disease affecting cultivated violets and generally known as spot. There is not less than a million dollars' worth of violet flowers sold every year in the United States, and were it not for the disease in question the amount would doubtless be increased 20 per cent. The annual loss from the disease, therefore, represents probably a money value of fully $200,000. In view of the general interest in violet culture and the importance of the knowledge of a means of preventing the disease, I respectfully recommend the publication of the paper as Bulletin No. 23 of this Division.

Respectfully,

B. T. Galloway,
Chief of Division.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

Page
Introduction 7
General appearance of the disease 8
Theories of the cause and treatment of the disease 9
  Weakness of the plants 9
  Improper soil conditions 10
  Improper conditions furnished the plants during the growing and flowering season 10
Fungous nature of the disease 10
Conditions favoring the development and spread of the disease 13
Susceptibility of varieties 14
Preventive measures 15
Explanation of plates 16

ILLUSTRATIONS.

Page.

Plate I. Healthy and diseased leaves of Marie Louise violets 16

II. Young plants of Marie Louise violet from the cutting bed showing spot on their leaves 16

III. A healthy and a naturally infected Marie Louise plant 16

IV. A healthy (control) and an artificially infected Marie Louise plant.. 16

V. Plate cultures of *Alternaria violæ* and mycelium and spores from a diseased leaf 16

VI. Development and germination of spores of *Alternaria violæ* and pure culture upon Lima bean 16

VII. Diseased and healthy leaves of California violet 16

SPOT DISEASE OF THE VIOLET.

INTRODUCTION.

The subject of this paper is one of the most widespread and destructive maladies known to attack the violet. The disease has been discussed in the florists' journals under a variety of names, such as leaf spot, leaf rust, leaf blight, smallpox, etc. More commonly, however, the trouble is known as the "violet disease," growers not generally recognizing the fact that there is more than one malady attacking the violet. The disease occurs throughout this country wherever the violet is grown, and is probably of American origin. The cultivation of the violet has been abandoned in many sections of the country on account of its ravages, while in others it has become necessary to adopt new methods of handling the plants during the growing season.

Five or six years ago, for example, 50,000 to 75,000 square feet of glass in the vicinity of Alexandria, Va., were devoted to the cultivation of this crop, but on account of the disease the industry has been practically abandoned. A large grower near Boston, Mass., was forced, a few years ago, to abandon growing stock plants at his place on account of this trouble. He had to have them grown for him during the summer, at considerable expense, in localities that were free or comparatively free from the disease. After transferring these plants to his place in the fall and setting them in the houses he experienced little or no difficulty in keeping them healthy during the remainder of the season. Many other instances of the destructive nature of this disease could be cited.

The large amount of florists' literature relating to this subject when collected and condensed was found by the writer to contain only a confused mass of contradictory opinions regarding both the cause and treatment of the disease. This is not strange to one familiar with the violet. All growers know the violet to be variable, seldom if ever behaving any two seasons alike. Practical growers recognize the fact that methods of handling the plants followed with little disease and good results during one season may, though rigidly adhered to, result in disease and failure the next. It is also a well-known fact that growers in the same section and in close proximity to one another often practice widely different methods in growing this crop, and yet the results obtained are practically the same. A novice in violet growing may have little or no difficulty the first few years in growing good flowers. After this, however, his troubles usually begin and failure more often than success crowns his efforts. Unless he is possessed of peculiar abilities and a determination to succeed a few years of reverses are sufficient to cause him to abandon the culture of violets and turn his attention to some other industry where the chances of success are at least equal to those of failure.

GENERAL APPEARANCE OF THE DISEASE.

Spot disease of the violet (*Alternaria violæ*) attacks the plants at any stage of their growth from the small unrooted cutting in the cutting bed to the mature plant in full flower. (See Pls. II, III, and IV.) Plants that are making a vigorous, rapid, but soft or succulent growth are most subject to the disease. The disease may occur on any portion of the plant above ground, but causes the greatest amount of loss when present upon the foliage. Its first appearance upon the leaves is characterized by small, definite, usually circular, greenish or yellowish white spots, resembling very much the bite or sting of an insect. They vary in size from dots scarcely perceptible to the unaided eye to spots a thirty-second of an inch or more in diameter. The light-colored central portion or point of infection is surrounded by a narrow ring of discolored tissue, usually black or very dark brown at first, but changing to a lighter shade as the spots grow older. (Pls. II, III, IV.) As the spot develops the central portion remains unchanged in appearance, while the tissues immediately surrounding it, either to one side or more frequently in a circle, become diseased by the ramifying growth of the mycelium of the fungus through this portion of the leaf. This usually takes place within a few hours after infection. The freshly diseased portion of the leaf at first presents a water-logged appearance, frequently being semi-transparent, and is lighter in color than the adjacent healthy tissue. The diseased portion around the central point of infection in a few days fades or bleaches to a yellowish or grayish white, sometimes to a pure white, the time depending somewhat upon the conditions of the weather. The development of the disease may stop at this point and the plants apparently entirely recover from its effects; in which event the diseased portions of the leaves after a few days separate from the healthy tissue and fall out, leaving the leaves full of holes. More frequently, however, the disease continues to develop in the parts of the leaf adjoining or surrounding those already diseased. These freshly diseased areas in turn pass through the same changes as the parts previously attacked. Unless checked by some means the disease continues to spread in this way until the entire leaf is destroyed. It is seldom, however, that a single spot upon a leaf develops to this extent. More frequently the leaf is attacked at a number of different points (Pls. I, II, III, IV), and as the disease progresses the spots become larger and one or more of them coalesce, forming large irregular areas or blotches upon the leaf. (Pls. I, II.) A well-developed spot of this disease therefore shows a light-colored central portion, the point of infection, partly or wholly surrounded by alternate rings of dark and light colored tissue, the lighter colored portions as a rule being very much broader and more conspicuous than the darker. (Pls. I, II.) The majority of these spots are usually free from fungous spores except under conditions peculiarly favorable to their development. Spores are produced, however, in great abundance upon most of them, especially upon the central or older portions of the spots, after the leaves have been placed in a saturated atmosphere for from twenty-four to forty-eight hours. It is frequently the case that spores are produced in sufficient numbers to be discernible by the unaided eye, but usually the aid of a hand lens or a microscope is necessary to determine their presence. The spores are borne in chains on dark brownish hyphæ that rise from the diseased surface. Pl. V, fig. 2, shows a photomicrograph of some of the mycelium and spores of this fungus taken from a diseased spot in a living leaf. The spores break from their attachment and separate from each other easily, and being very small and light they are carried around by currents of air and finally settle upon other leaves.

THEORIES AS TO THE CAUSE AND TREATMENT OF THE DISEASE.

Perhaps no subject relating to floriculture has received more attention in the floricultural and horticultural journals during the past eight or ten years than the disease in question. The most varied opinions have been expressed in regard to it, and the explanations advanced as to its cause and the possible course of treatment are numerous. Some of the more important of these hypotheses are given here.

WEAKNESS OF THE PLANTS.

Some writers claim that the plants are of necessity weakened by being forced during the winter into heavy flower production, and that the taking of cuttings from such plants, and the rooting and forcing of them in the same way from year to year has resulted in producing a weak strain peculiarly susceptible to injury of all kinds. They recommend fall propagation to secure strong, vigorous, healthy wood before the plants are weakened by flowering. The cuttings, after being rooted in clean, sharp sand, are transplanted into thumb-pots or into flats and carried through the winter in a house or in frames, where the temperature is kept as low as possible, not allowing the plants to freeze, however. By this treatment the plants are given a rest, which is believed by many to be necessary to strong, vigorous growth. While growers generally admit that slightly better results are usually obtained by this treatment than by the one generally practiced, they are, as a rule, of the opinion that the benefits derived will not justify the expense necessary to carry the young plants through the winter in good condition for spring planting. This is an important problem, the practical solution of which would no doubt prove of great value to all interested in the cultivation of the violet. We have this work under way at the present time, and hope in a few years to obtain some interesting results.

IMPROPER SOIL CONDITIONS.

Other writers claim that the disease is due to improper soil conditions. The soil is either too heavy or too light in texture, and as a consequence holds, or gives up, too much or too little moisture, or contains too much or too little plant food. They advise selecting soil suited in every way to the best growth and development of the plants.

Since good soil is one of the prime factors governing strong, vigorous, healthy plant growth, their advice is good, but extremely difficult to follow. The question of securing proper soil is one of the most perplexing with which the grower has to contend, requiring judgment that can be gained only by many years of practical experience.

IMPROPER CONDITIONS FURNISHED THE PLANTS DURING THE GROWING AND FLOWERING SEASON.

Still others attribute the disease to improper methods employed during the growth of the plants, such as growing them in the open field, where they are exposed to drought, rains, dews, and the direct rays of the sun during the summer, and lack of attention to properly heating, ventilating, and fumigating the houses and to cultivating, watering, and cleaning the plants. As a remedy they propose furnishing the necessary conditions for vigorous, healthy plant growth at all times. This is a good doctrine, but begs the question.

FUNGOUS NATURE OF THE DISEASE.

Over four years ago the writer succeeded in producing upon violet leaves spots that were in every way identical with those above described by spraying the leaves with distilled water to which spores of the fungus *Alternaria violæ* had been added. Since that time he has proved by numerous laboratory and greenhouse experiments (details of which will appear in the following pages) that the so-called "spot disease" of the violet is unquestionably due to the attacks of this fungus. Other fungi, *Cercospora violæ* Sacc., *Phyllosticta violæ* Desm., *Septoria violæ* Westd., etc., are known to attack the violet, producing upon the leaves spots very similar in outline and appearance to those caused by *Alternaria violæ* (with which they are often confused), but in the writer's experience in the study of the violet and its diseases he does not recall a single instance where these fungi have come to his attention as causing any serious trouble. It is possible, however, for them to do considerable damage under conditions peculiarly favorable to their development.

Ninety-five per cent of all the specimens of the so-called violet disease received at the Division laboratory during the past four or five years were found, upon careful microscopical examination, to contain spores of the particular fungus mentioned.[1]

The fungus was isolated by agar poured cultures in Petri dishes, and comparatively little difficulty was experienced in securing pure cultures for inoculation experiments. The growth and development of the fungus on artificial media is, as a rule, quite rapid, normally producing spores in from four to six days after the sowing of the spores or the transferring of a single germinating spore from one plate culture to another.

The growth of the fungus in agar is normally in concentric rings, each ring marking the amount of growth made in twenty-four hours (Pl. V, fig. 1). The color of the fungus growing on agar before spore formation is grayish white (Pl. V, fig. 3). Spore production begins at the center on the older growth, and gradually extends outward, until the entire surface of the colony is covered with a dense mass of olivaceous spores. The fungus grows well on other culture media, especially young lima bean pods (Pl. VI, fig. 18).

The first inoculation experiment with *Alternaria violæ* was made February 12, 1896. Two plants of Marie Louise violet, in 4-inch pots, were removed from the Department greenhouse to the laboratory. They were quite uniform in size and, as far as could be ascertained by observation, entirely free from disease. Plant No. 1 was sprayed with sterile distilled water and placed under a bell jar in a saturated atmosphere, where it was kept. Plant No. 2 was sprayed with sterile distilled water in which spores from a pure culture of *Alternaria violæ* had been sown, and was then placed under the same conditions as plant No. 1. The temperature of the laboratory at the beginning of the experiment—3.30 p. m.—was about 80° F. The following notes, made during the progress of the experiment, are descriptive of the results obtained:

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[1] SCIENTIFIC DESCRIPTION OF THE FUNGUS.

Alternaria violæ Galloway and Dorsett.

Amphigenous, but especially epiphyllous, olivaceous, velutinous, on light-colored subcircular definitely limited spots 2–4 mm. in diameter, extending into arid patches 10–12 mm. in diameter, which show one or more dark concentric lines; spore-bearing hyphæ fasciculate, erect, pale olivaceous, septate, simple, 4 by 25–30 μ; conidia borne at or near tips of the hyphæ, catenulate, clavately flask-shaped, muriform, strongly constricted at the septa, which are variable in number, olivaceous, 10–17 by 40–60 μ exclusive of isthmus, which is 3–5 by 3–25 μ.

Dr. Gino Pollacci has described and figured (Atti del R. Inst. Bot. dell'Univ. di Pavia (Laboratorio crittogamico) Ser. II, Vol. V, pp. 1-2, Pl. VII, figs. 1-5, 1897) a spot disease of violet occurring in Italy which is due to a fungus which he names *Macrosporium violæ*. If his description and drawings are correct the two diseases are quite distinct.

February 14, 1896, 9.30 a. m. Plant 1 apparently in a perfectly healthy condition, leaves covered with moisture, but showing no ill effects from the spraying or from being kept in a saturated atmosphere. Plant 2 badly diseased, nearly every leaf showing one or more spots of infection, which are in every particular identical with the first stages of the disease as naturally produced.

February 15, 1896, 9.30 a. m. Plant 1 still remains healthy and apparently uninjured by the treatment. On plant 2 the disease is progressing rapidly. There is a peculiarly disagreeable odor present when the bell jar is removed that is not noticeable under the same conditions with plant 1. This odor, so far as I am able to judge, is identical with that noticed with plants suffering from an attack of the disease under normal conditions. This odor is one of the characteristics of the disease, and its presence in the house, frame, or field is usually the first intimation the grower has of the presence of the disease among his plants.

February 19, 1896. Plant 1 still healthy and apparently in good condition. The spots on plant 2 are a little further developed and resemble more closely those produced under natural conditions.

A striking example of the results obtained by artificial inoculations of violets with this fungus is shown in Pl. IV. The two plants shown in this photograph received the same treatment as that given the two plants in the experiment described. The plants were sprayed at 3.30 p. m., August 26, 1896, and examined first at 2.30 p. m., August 27, 1896, just twenty-three hours after treatment. At this time plant No. 1 appeared free from disease, and showed no ill effects whatever from the treatment. On the contrary each leaf of plant No. 2, with one or two exceptions, showed from 1 to 30 or more spots of the disease, which were in every way identical with those produced on plant No. 2 in the previous experiment.

The plants were photographed August 31, 1896. At this date plant No. 1 was apparently free from disease, while the disease on plant No. 2 had made considerable progress and the spots were gradually assuming the normal colorings which are characteristic of this disease.

In these spots thus produced a careful microscopic examination demonstrated the presence of the mycelium of the fungus, and subsequent observation showed that the fungus pushed through to the surface of the spots and fruited, whenever the leaf was put under a bell jar in moist air, exactly as it did on spots occurring naturally. The disease was again produced in healthy plants by inoculation with the spores thus formed.

That the spots produced upon violet leaves by artificial inoculations with spores of *Alternaria violæ* closely resemble those occurring naturally can be readily determined by comparing Pl. III, which is a photograph of a naturally diseased and a healthy plant from greenhouses at Garrett Park, Md., December 18, 1897, with Pl. IV, which is a photograph of a healthy and an artificially infected plant. The similarity of the spots is, however, more strikingly shown in Pl. I. Fig. 1 is a healthy leaf; fig. 2 is a diseased leaf from a plant naturally infected with the disease, while leaves 3 and 4 were taken from the diseased plant shown in Pl. IV, which was artificially infected with the disease.

Spores of the fungus *Alternaria violæ* sown in water in a van Tieghem cell and kept at a temperature of 65° to 80° F. germinate readily in from one and a half to three hours. Fig. 9, Pl. VI, is a camera lucida drawing of a group of spores that were sown in distilled water and placed in a van Tieghem cell at 10 a. m., January 15, 1898. The dotted lines at right angles to several of the germ tubes mark the amount of growth made by them between the time of sowing and the time noted; all subsequent growth and the production of all germ tubes not marked with a dotted line occurred between 11.55 a. m. and 2.10 p. m. the same day. Figs. 1 to 6 show a camera lucida drawing of a group of *Alternaria* spores in distilled water just previous to being placed in a van Tieghem cell, and figs. 7 and 8, two spores in the same sowing, nineteen hours later. Figs. 11, 13, and 14 show spores that were sown in distilled water in a van Tieghem cell at 10.20 a. m., August 19, 1898, at which time they showed no signs of germinating. Four hours later, however, at 2.20 p. m., the time at which the drawings were made, the number of germ tubes had developed as indicated. Fig. 12 is a second drawing of fig. 11, made twenty minutes later. Fig. 10 is a camera lucida drawing of two spores from four to six hours after being placed in distilled water. Figs. 15, 16, and 17 show the chain formation of spores and their attachment to the mycelium. This drawing was made from a pure plate culture of the fungus.

Numerous greenhouse and laboratory experiments under strict control conditions have confirmed these results, and show that spot disease of the violet is due directly to the attack of the parasitic fungus *Alternaria violæ*, and not to any of the other causes suggested. Indirectly, however, other conditions may have their effect. Any one or a combination of all of the conditions included in the various theories advanced may cause the plants to become susceptible to the attacks of the fungus.

CONDITIONS FAVORING THE DEVELOPMENT AND SPREAD OF THE DISEASE.

The conditions favoring the development and spread of the fungus may be considered under two heads, viz, natural conditions and artificial conditions.

Among natural conditions those of the damp, warm, cloudy weather of the summer season are the most difficult to modify or control.

Conditions of this nature are almost invariably present during the months of August and September. The days are long and usually hot and dry, followed, as a rule, by cool, moist nights. The plants at this time are subjected to extreme changes, viz, from the hot, dry atmosphere during the day, which frequently causes them to become wilted and remain so for several hours, to the cool, moist atmosphere of the night, which causes them to become excessively turgid. Conditions of this kind induce a rapid, weak, soft, or succulent growth of the plants which is particularly subject to disease and at the same time favors the germination and development of the spores of the fungus. It is at this season of the year, as a rule, that the spot disease is most abundant and destructive. This is the time for great vigilance, and every condition influencing plant growth must be made as favorable as possible to a hardy, healthy growth which will be able to withstand the attacks of disease. The grower who is able to accomplish this and tide his plants over this critical period of their growth in a comparatively healthy condition is fortunate, and, as a rule, has little to fear from the disease during the remainder of the season.

Artificial conditions include those wholly or in part under the control of the grower. They are too often neglected, resulting as a rule in disease and consequent loss and discouragement. They may be enumerated as follows:

(1) Not keeping the houses or frames clean, fresh, and sweet by frequently repairing and painting them and by removing and destroying rubbish of all kinds as soon as it appears.

(2) Not keeping the plants clean and in the best possible growing condition at all times.

(3) Not selecting stock from strong, vigorous plants that have been entirely free from disease.

(4) Not being careful to select only strong, vigorous, healthy stock from the cutting bed for planting in the spring.

(5) Not giving the proper attention to the selection and preparation of the soil, to the date and method of planting, and to the care and cultivation of the plants during the growing season.

(6) Not giving due consideration to the several varieties and their adaptability to the soil and location in which they are to be grown.

SUSCEPTIBILITY OF VARIETIES.

While the susceptibility of the plant to disease depends largely upon the way in which it has been grown, still, as a whole, some varieties are more susceptible than others; Marie Louise, for example, even under conditions most favorable to growth, is more subject to injury from spot than is Lady Hume Campbell. The former variety can be grown to perfection only under the most favorable conditions, but when thus grown it has no equal for size, color, and excellency of flower. The hardier, more resistant, and more prolific variety Campbell stands next to Marie Louise in quality of flowers, lacking only the deep rich color of the latter. The single varieties are as a rule more resistant than the double, though occasionally they are seriously affected. (Plate VII.)

PREVENTIVE MEASURES.

So far as we are aware there is at present no effective remedy for this disease when it has gained a foothold. The principal fungicides in common use for the prevention and check of plant diseases have frequently been tried for this trouble, but with varying results. The experiments of the Division in spraying violets with some of the more important of these, among them Bordeaux mixture and ammoniacal solution of copper carbonate, seem to show that they possess little or no value in preventing the disease, while on the other hand they render the foliage worthless for bunching with the flowers, and thus occasion considerable loss and inconvenience. From the writer's experience and that of many others it would seem that the solution of this problem of controlling the disease lies in preventing it by giving careful attention to the production of vigorous, healthy, plant growth rather than in attempting to check the trouble after it has once gained a foothold.

The successful growing of violets free from disease and the production of flowers of the best quality are governed by a number of factors which must be kept in mind. The principal rules which should govern the grower are the following:

(1) Study carefully the behavior of the plants under the varying conditions surrounding them. Endeavor by modifying these conditions, when necessary, to secure plants of ideal development. Set the standard of excellence high and be satisfied with nothing short of its attainment.

(2) Grow the plants during the entire season where they can be given the conditions necessary for making a vigorous, healthy growth, and where they can be protected at all times from conditions likely to induce disease.

(3) Keep the houses or frames clean, sweet, and in perfect condition for growing healthy plants, by repairing and painting them when necessary, and by removing and destroying all rubbish likely to harbor vermin or disease.

(4) Propagate only from healthy, vigorous stock of known parentage at the season most favorable to the plants.

(5) Select each spring none but perfectly healthy, vigorous plants from the rooted cuttings for planting into the houses or frames. Old plants are sometimes carried over, and occasionally yield a large crop of flowers. They are not as reliable as the young plants, however, and are much more liable to all kinds of disease. The best growers rarely use them if it is possible to secure strong, healthy young plants for spring or early summer planting.

(6) Keep the plants clean of yellow, dead, or dying leaves, being careful to destroy them after removing them from the plants.

(7) Keep the plants free from insects and other animal pests.

(8) Give careful attention to ventilating, heating, and shading the houses or frames and to watering, cleaning, and cultivating the plants.

(9) Renew the soil in the beds each season before setting in the young plants by removing from eight to twelve inches of the surface soil and replacing it with that freshly prepared.

(10) Set the young plants early in the spring in the beds where they are to remain during the season, so that they may get well established before the hot, dry weather of summer makes its appearance.

Careful attention given to the above directions for a number of years will, it is believed, result in the production of a strain of plants that are not only practically disease resistant, but are also ideal as regards regularity and symmetry of growth, length, and strength of flower stems, and yield, size, substance, and quality of flowers produced.

EXPLANATION OF PLATES.

Plate I. Healthy and diseased leaves of Marie Louise violet. (Natural size.) Fig. 1, healthy leaf. Fig. 2, naturally infected leaf. Figs. 3 and 4, artificially infected leaves from the diseased plant shown in Plate IV.

II. Young plants of Marie Louise violet from the cutting bed, showing spot on the leaves.

III. Fig. 1, healthy plant of Marie Louise violet for comparison. Fig. 2, diseased plant (natural infection).

IV. Fig. 1, healthy plant of Marie Louise violet (control). Fig. 2, diseased plant (artificial infection).

V. Fig. 1, growth of the fungus in eleven days from a single spore on an agar plate. Fig. 2, photomicrograph of mycelium and spores of *Alternaria violæ* from violet leaves. Fig. 3, pure plate culture of *Alternaria violæ*.

VI. Figs. 1 to 6, inclusive, show spores as they appear when brushed from a diseased spot. Figs. 7 and 8, some of the same spores after nineteen hours in distilled water in a van Tieghem cell. Fig. 9, a group of germinating spores. The length of the germ tubes at the time of the first examination is indicated by the dotted lines, and time marked; all subsequent growth of these tubes occurred, and all unmarked tubes developed, between the time marked and 2.10 p. m. Fig. 10, two connected spores germinating at several points after being about four hours in distilled water. Fig. 11, germinating spore. Fig. 12, the same spore twenty minutes later. Figs. 13 and 14, germinating spores. Figs. 15, 16, and 17, spores produced on agar, showing manner of attachment to mycelium and chain formation of spores. Fig. 18, pure culture of *Alternaria violæ* on sterilized lima bean. The darker part of the culture is thickly covered with spores; the white marginal portions are young growing mycelium.

VII. Fig. 1, healthy leaf of California violet. Figs. 2 and 3, diseased leaves of California violet.

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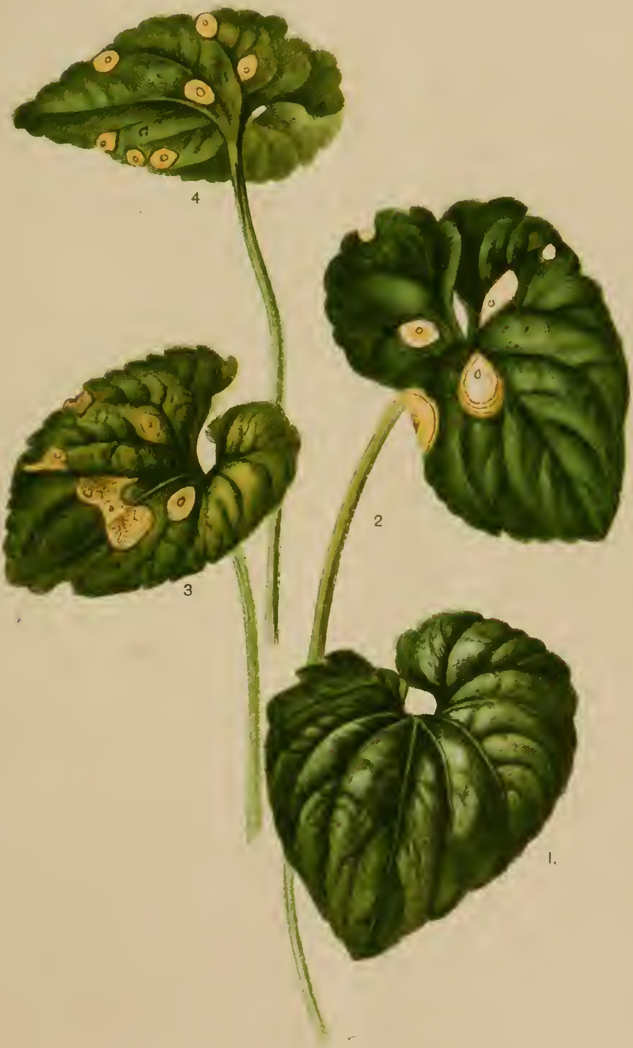

D. G. PASSMORE.

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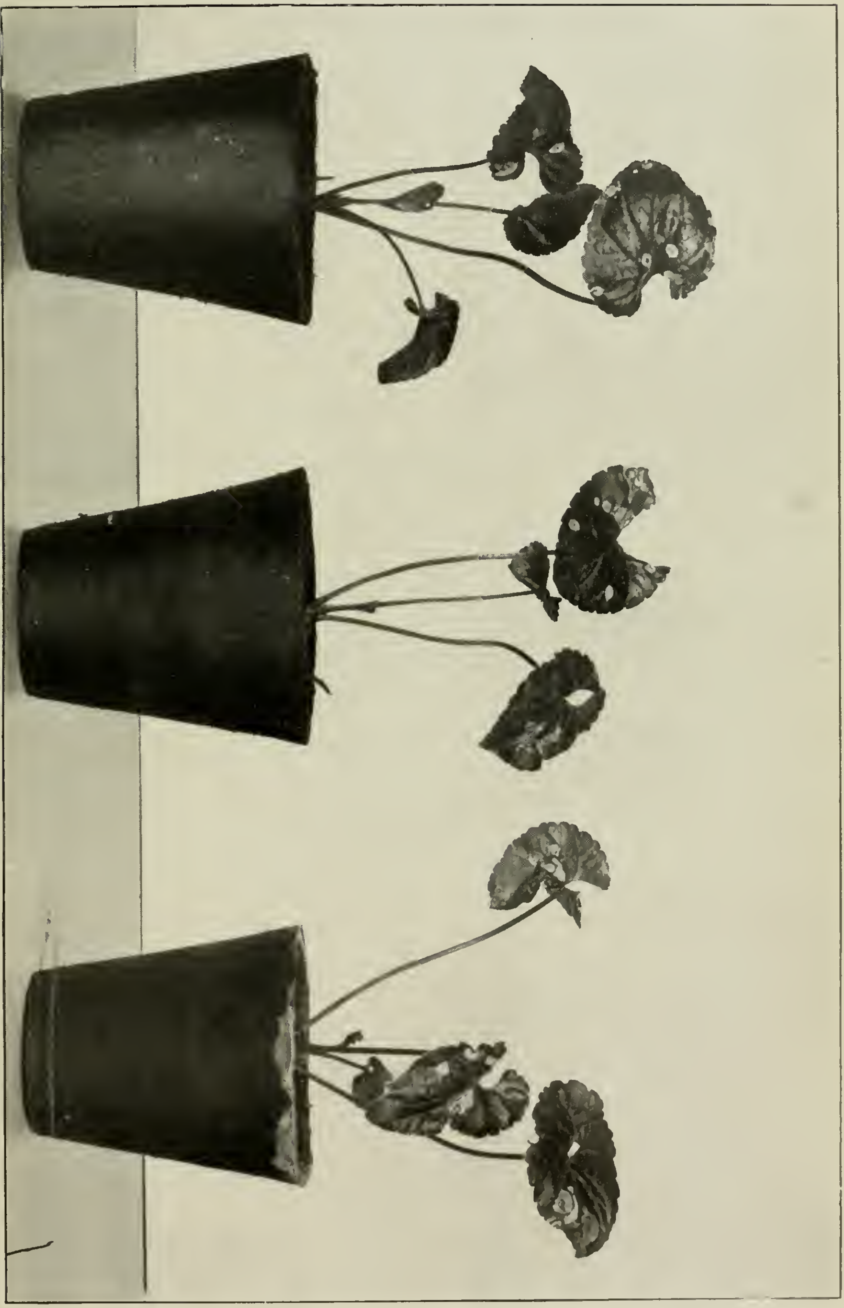
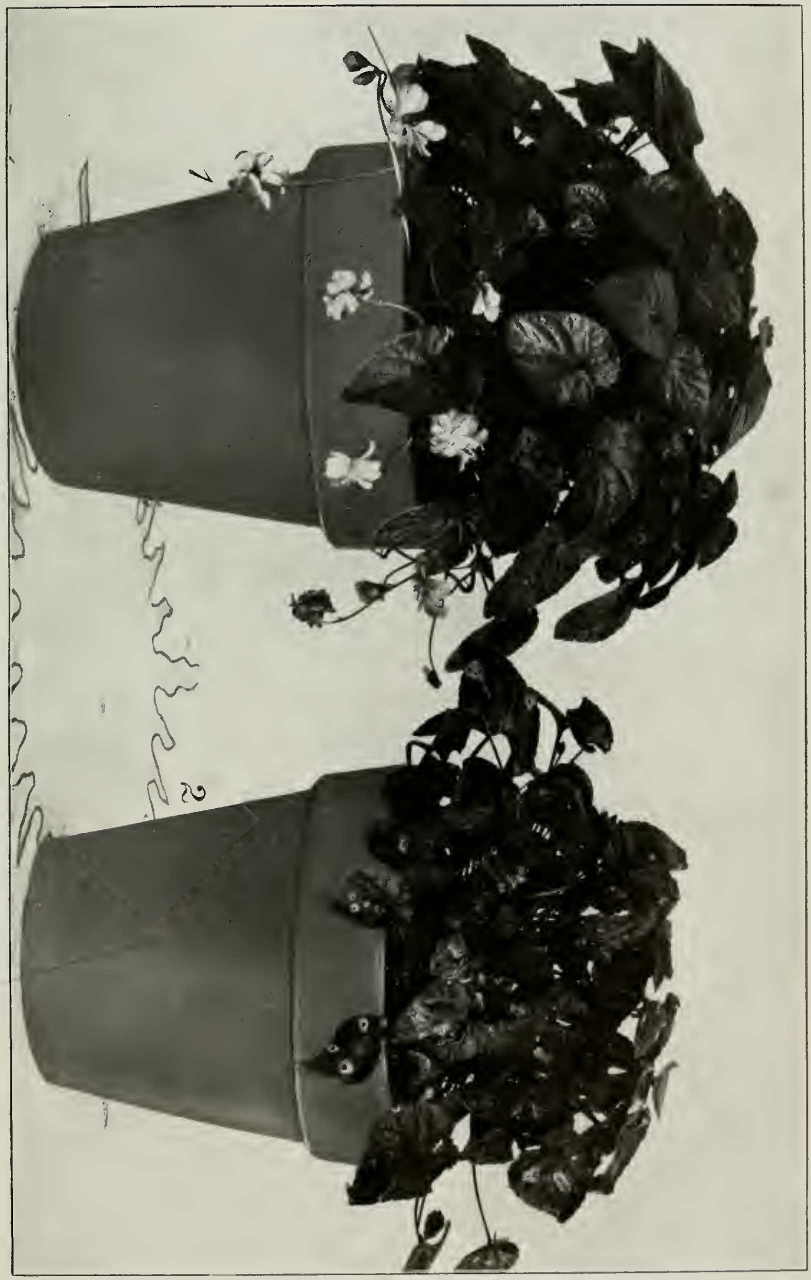
HEALTHY AND DISEASED LEAVES OF MARIE LOUISE VIOLET.

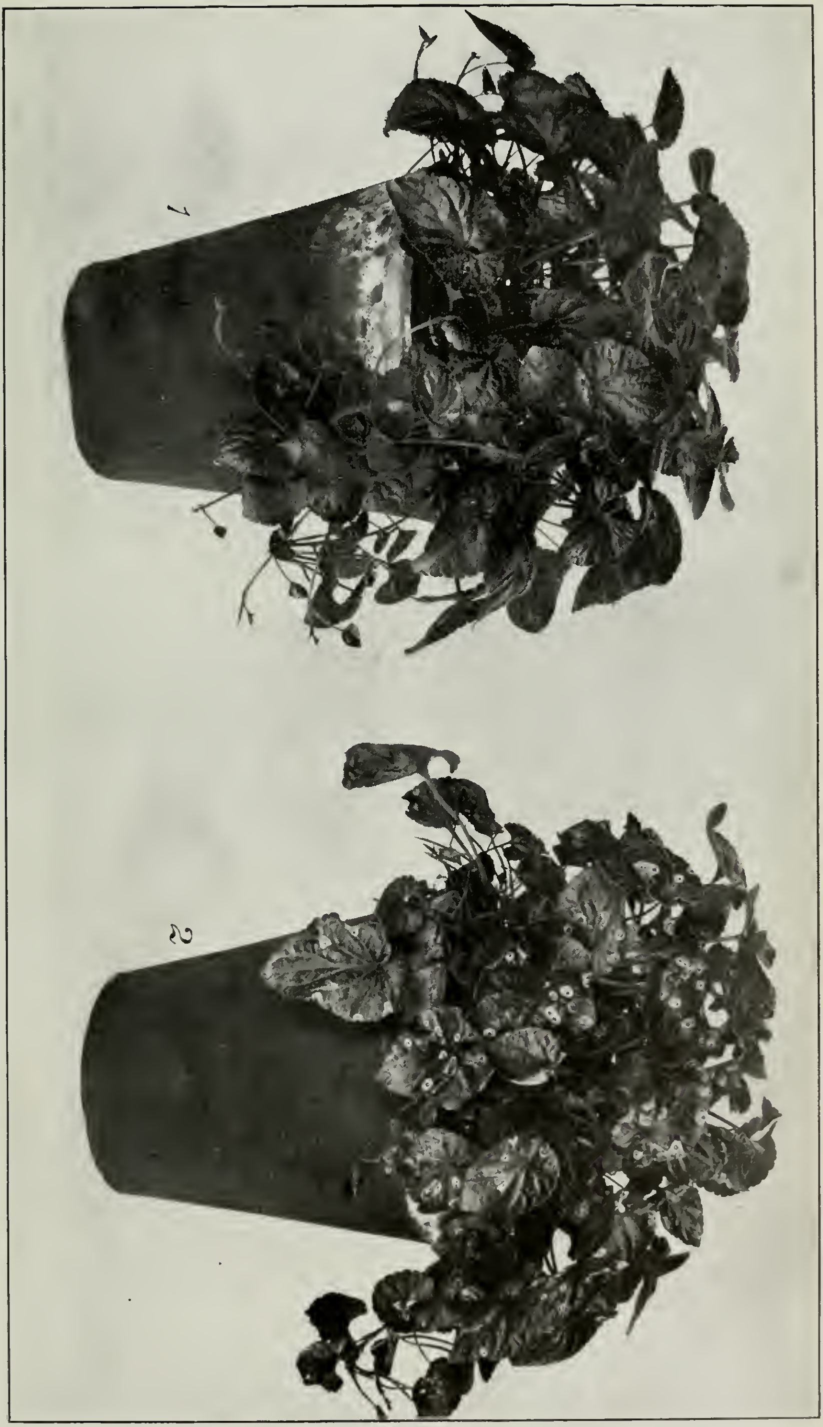


YOUNG PLANTS OF MARIE LOUISE VIOLETS FROM THE CUTTING BED, SHOWING SPOT ON LEAVES.



A HEALTHY AND A NATURALLY INFECTED MARIE LOUISE PLANT.

Plate IV.



A Healthy (Control) and an Artificially Infected Marie Louise Plant.

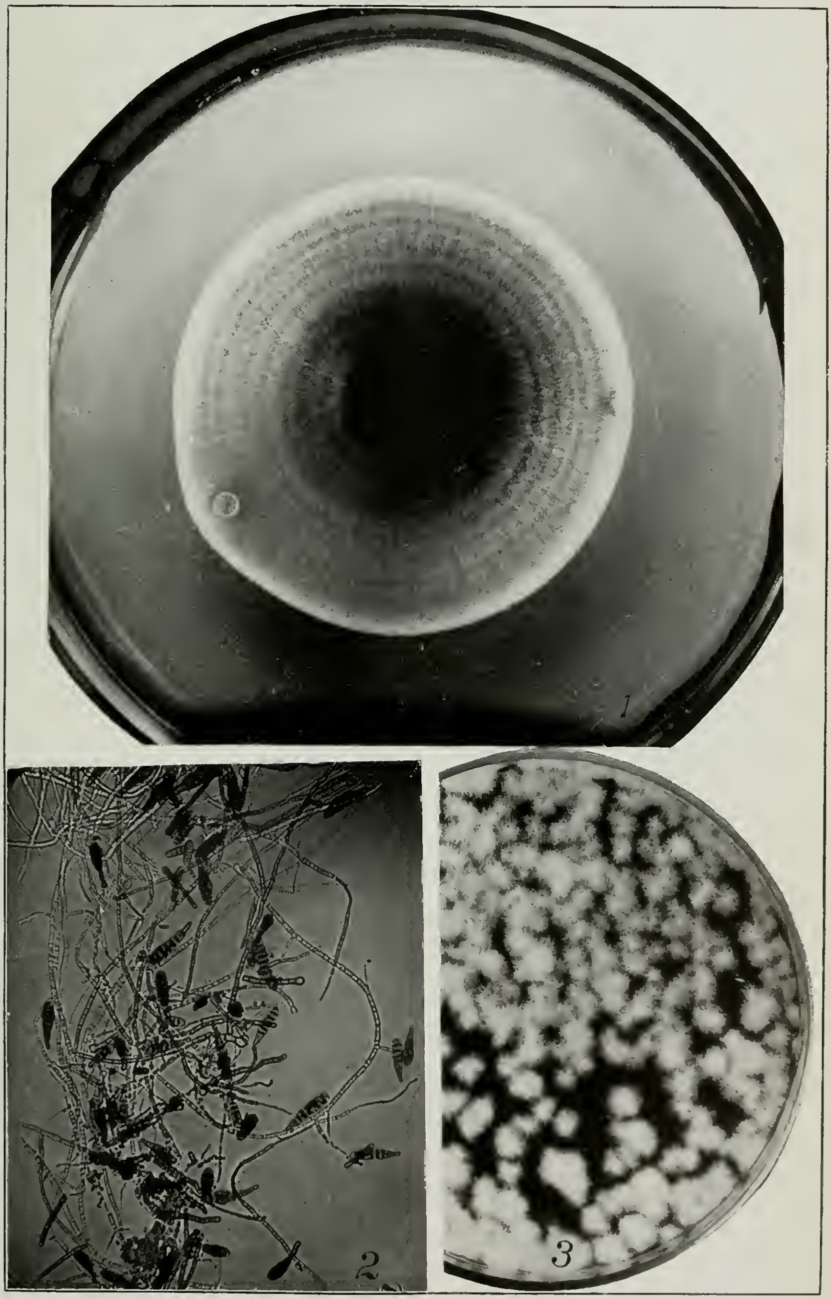
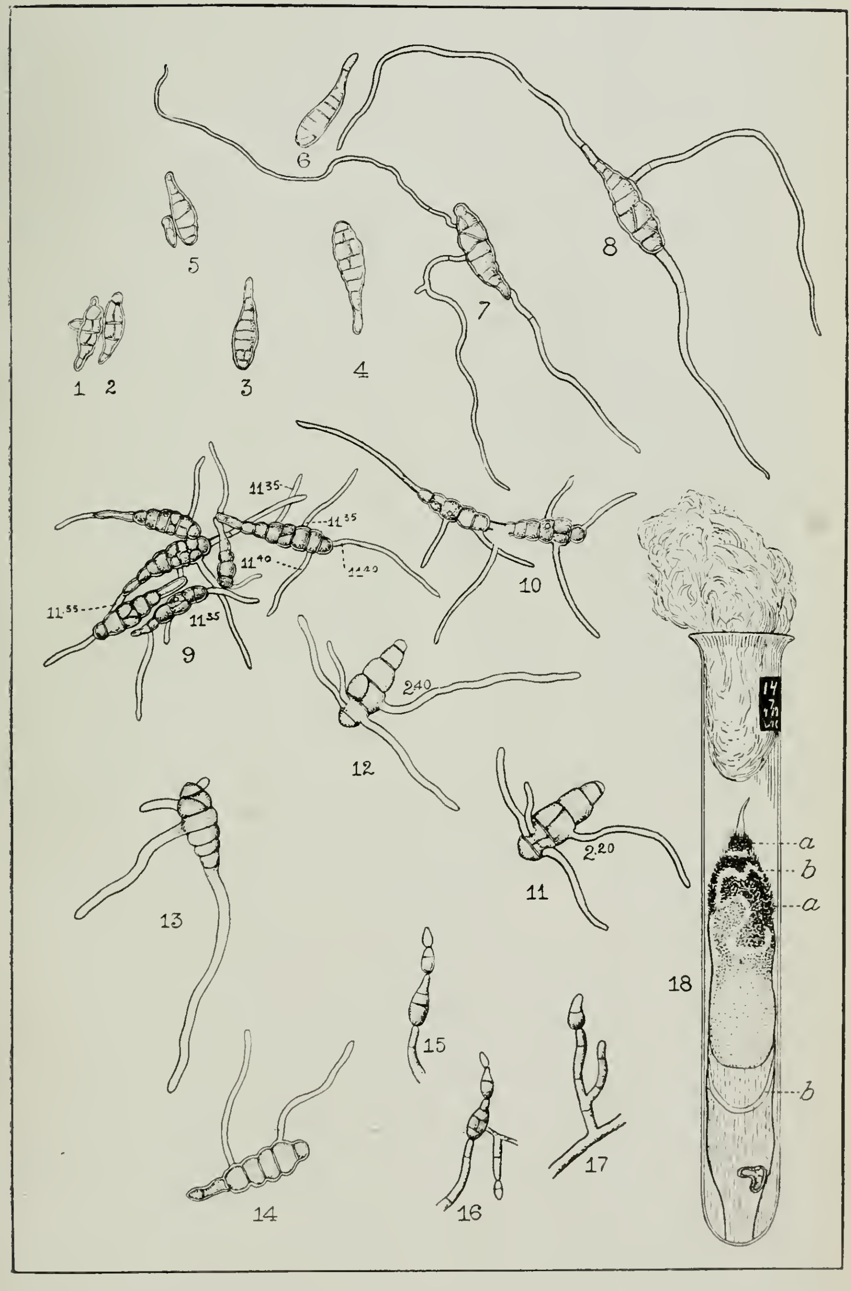


PLATE CULTURE OF ALTERNARIA VIOLÆ, AND MYCELIUM AND SPORES FROM A DISEASED LEAF.

PLATE VI.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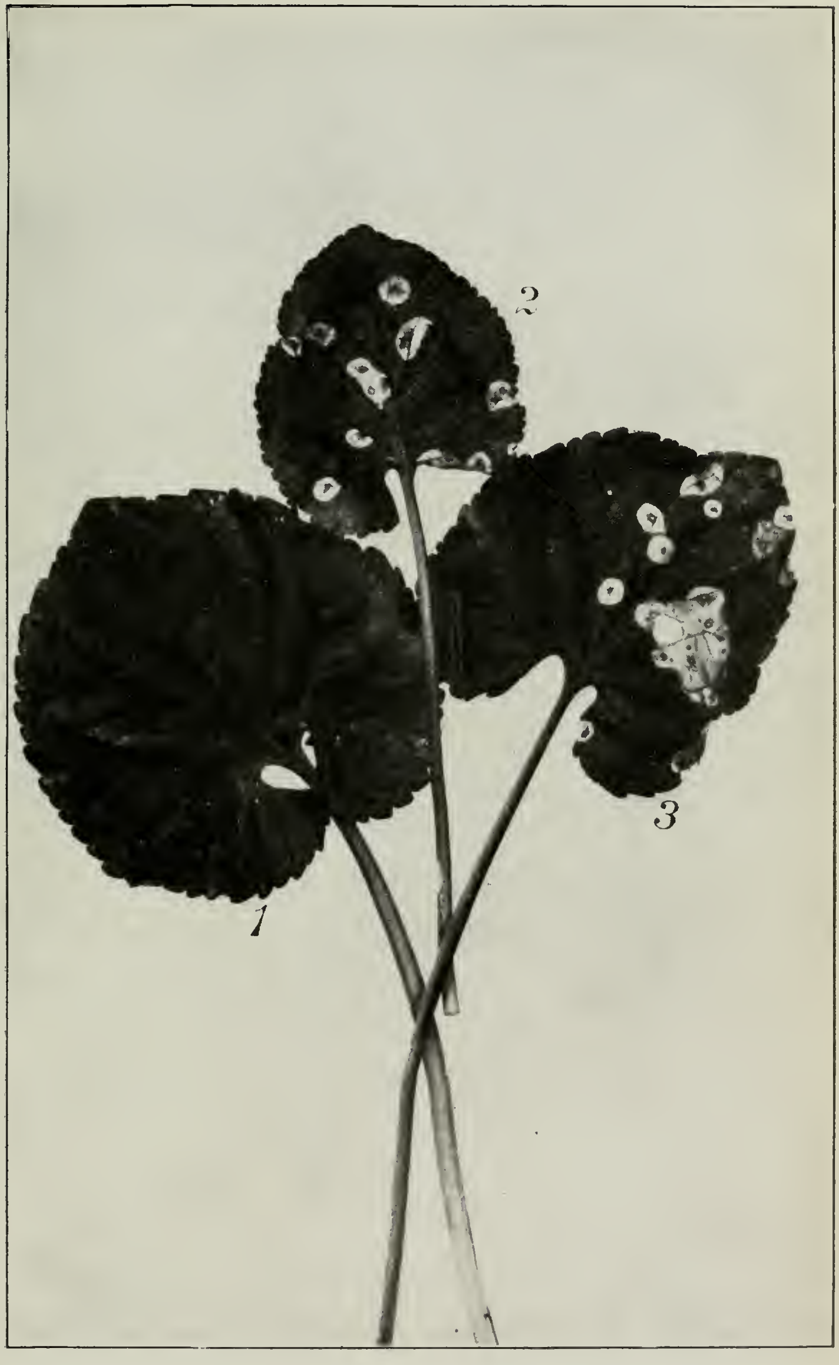
DEVELOPMENT AND GERMINATION OF SPORES OF ALTERNARIA VIOLÆ AND PURE CULTURE UPON LIMA BEAN.



DISEASED AND HEALTHY LEAVES OF CALIFORNIA VIOLET.

BULLETIN No. 24.
V P. P.—77.

U. S. DEPARTMENT OF AGRICULTURE,

DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY.

B. T. GALLOWAY, Chief.

THE BASIS FOR THE IMPROVEMENT OF AMERICAN WHEATS.

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BY

MARK ALFRED CARLETON,
Cerealist, Division of Vegetable Physiology and Pathology.

ISSUED DECEMBER 10, 1900.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1900.

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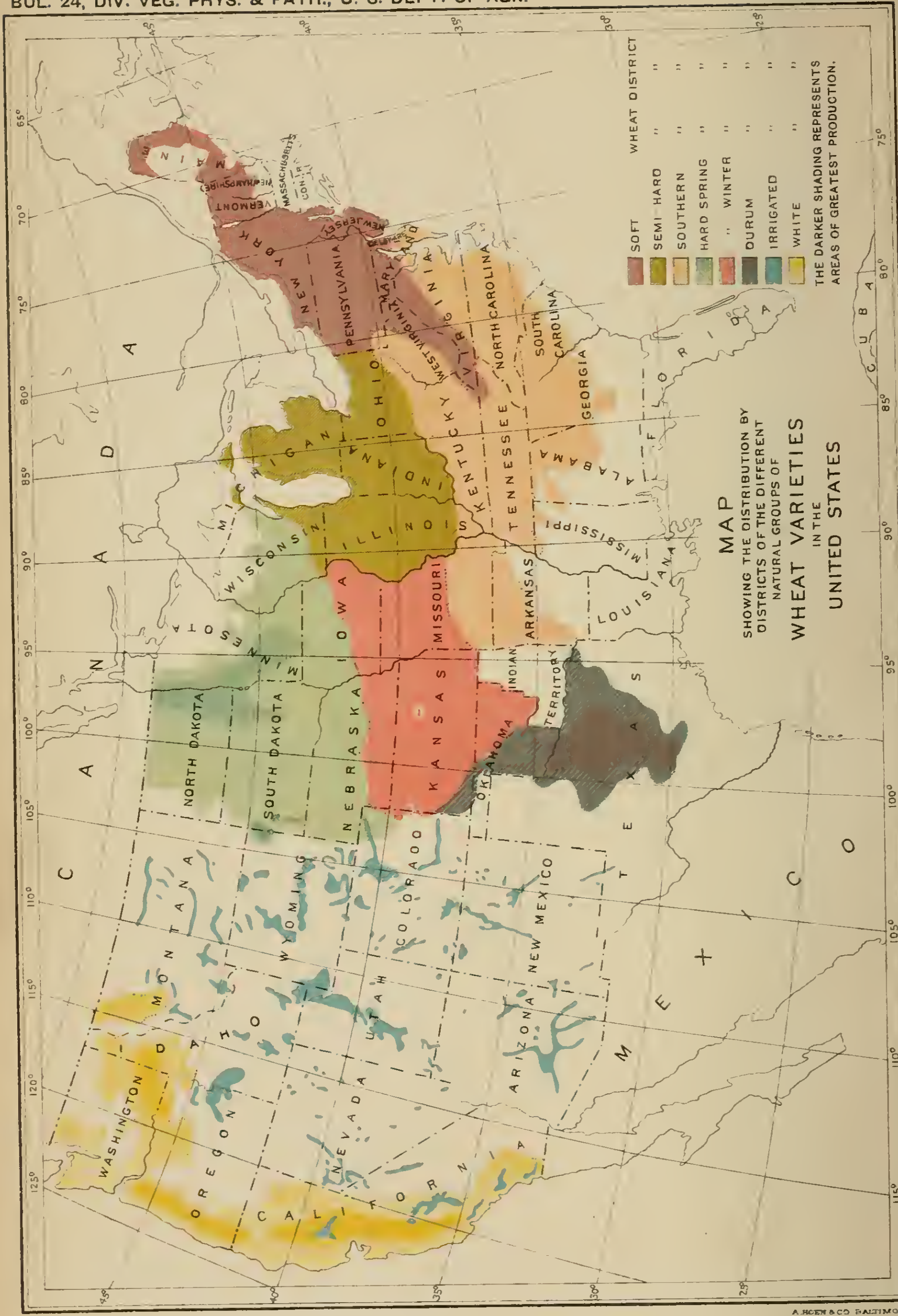

MAP
SHOWING THE DISTRIBUTION BY
DISTRICTS OF THE DIFFERENT
NATURAL GROUPS OF
WHEAT VARIETIES
IN THE
UNITED STATES

SOFT WHEAT DISTRICT
SEMI-HARD " "
SOUTHERN " "
HARD SPRING " "
" WINTER " "
DURUM " "
IRRIGATED " "
WHITE " "
THE DARKER SHADING REPRESENTS AREAS OF GREATEST PRODUCTION.
CANADA
MAINE
NEW HAMPSHIRE
VERMONT
MASSACHUSETTS
CONN.
R.I.
NEW YORK
NEW JERSEY
PENNSYLVANIA
DELAWARE
MARYLAND
WEST VIRGINIA
VIRGINIA
NORTH CAROLINA
SOUTH CAROLINA
GEORGIA
FLORIDA
ALABAMA
MISSISSIPPI
LOUISIANA
TENNESSEE
KENTUCKY
OHIO
INDIANA
ILLINOIS
MICHIGAN
WISCONSIN
MINNESOTA
IOWA
MISSOURI
ARKANSAS
INDIAN TERRITORY
OKLAHOMA
TEXAS
KANSAS
NEBRASKA
SOUTH DAKOTA
NORTH DAKOTA
MONTANA
WYOMING
COLORADO
NEW MEXICO
ARIZONA
UTAH
NEVADA
IDAHO
WASHINGTON
OREGON
CALIFORNIA
MEXICO
CUBA
65° 70° 75° 80° 85° 90° 95° 100° 105° 110° 115° 120° 125°
45° 40° 35° 30° 25°

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BULLETIN NO. 24. V. P. P.—77.

U. S. DEPARTMENT OF AGRICULTURE,

DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY.

B. T. GALLOWAY, Chief.

THE BASIS FOR THE IMPROVEMENT OF AMERICAN WHEATS.

BY

MARK ALFRED CARLETON,
Cerealist, Division of Vegetable Physiology and Pathology.

ISSUED DECEMBER 10, 1900.

1862 1889

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1900.

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Division of Vegetable Physiology and Pathology,
Washington, D. C., July 19, 1900.

Sir: I have the honor to transmit herewith, and to recommend for publication as Bulletin No. 24 of this Division, the manuscript of a paper by Mr. M. A. Carleton, on The Basis for the Improvement of American Wheats. During the past ten years this Division has had under investigation a number of problems connected with cereal production, and in order to carry on this work intelligently it has been necessary to make a careful study of the wheat industry generally. To this end a thorough survey of the field has been made, and the results are brought together here. The bulletin will prove especially valuable as showing the lines along which further work must be carried on. Part of this work is already under way, and other lines will be taken up as rapidly as the means at hand will permit.

Respectfully,

B. T. Galloway,
Chief of Division.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

Page.
Introduction 7
Personal explorations 9
Characteristics and needs of the several wheat districts of the United States 9
  General needs of all the districts 10
    Yielding power 10
    Early maturity 11
  Soft wheat district 12
  Semihard winter wheat district 13
  Southern wheat district 14
  Hard spring wheat district 15
  Hard winter wheat district 17
  Durum wheat district 18
  Irrigated wheat district 20
  White wheat district 22
Sources for desirable qualities 25
  Characteristics of botanic groups of wheat 26
    Common bread wheats (*Triticum vulgare*) 26
    Club or square head wheats (*T. compactum*) 28
    Poulard wheats (*T. turgidum*) 29
    Durum wheats (*T. durum*) 30
    Polish wheats (*T. polonicum*) 32
    Spelt (*T. spelta*) 33
    Emmer (*T. dicoccum*) 34
    Einkorn (*T. monococcum*) 35
  Geographic groups of wheats 36
Improvements accomplished 37
  Introduction of new varieties 38
  Work of the Department 40
Wheat breeding 63
  Improvement by selection 64
  Improvement by hybridization 69
Summary 77

ILLUSTRATIONS.

PLATES.

Page.

Frontispiece. Map showing the distribution by districts of the different natural groups of wheat varieties in the United States.

Plate I. Wheat field near Mount Vernon, Va 12

II. Fig. 1, Wheat fields of the Red River Valley near Grand Forks, N. Dak. Fig. 2, Self-binders at work near Grand Forks, N. Dak 16

III. Fig. 1, Field of wheat on "Tule" lands near Stockton, Cal. Fig. 2, Steam combined harvester-thresher harvesting on "Tule" lands near Stockton, Cal 22

IV. Fig. 1, Bags of wheat just harvested on the Bidwell estate, Chico, Cal. Fig. 2, Wheat field near Tehama, Cal 22

V. Fig. 1, Harvesting with the combined harvester-thresher near Walla Walla, Wash. Fig. 2, Wheat fields before and after harvesting, near Walla Walla, Wash 24

VI. Fig. 1, Combined harvester-thresher at work near Walla Walla, Wash. Fig. 2, Harvesting with the wide-cut binder near Colfax, Wash 28

VII. Spelts and Einkorns in experimental plats at Garrett Park, Md 34

VIII. Fig. 1, Group of Russian wheats in experimental plats at Garrett Park, Md. Fig. 2, Experimental wheat plats at Garrett Park, Md., showing earliness of King's Jubilee 62

IX. Hybrid wheats, Early Arcadian and Diamond Grit, shown by the side of the parent varieties 72

X. A composite cross by the Gartons, showing samples of the progeny of the last cross 74

TEXT FIGURES.

Fig. 1. Diagram showing pedigree of Early Genesee Giant Wheat 71

2. Diagram showing pedigree of one of the Gartons' hybrid wheats 74

3. Diagram showing pedigree of one of the Gartons' hybrid wheats 75

4. Diagram showing pedigree of one of Farrer's hybrid wheats 76

5. Diagram showing hypothetical cross of wheat and spelt 76

THE BASIS FOR THE IMPROVEMENT OF AMERICAN WHEATS.

INTRODUCTION.

In 1894 the Division of Vegetable Physiology and Pathology began experiments on an extensive scale to test the comparative rust resistance of different varieties of cereals, especially wheat. This work was carried on for three seasons at Garrett Park, Md., Salina, Kans., and Manhattan, Kans., respectively. An account of the results of this work has already been published,[1] so that it is unnecessary to refer to them in detail here. Suffice it to say that in the course of the work it became apparent that constant rust resistance is not to be obtained among the ordinary bread wheats known at present, though on an average a few such varieties are fairly resistant during a long period of years. By the results obtained it was rendered highly probable that this quality must be bred into a variety either by rigid selection of the most resistant individuals of that variety or by crossing with resistant varieties of other wheat groups and selecting from the resultant progeny such types as combine in the highest degree the usual qualities of the bread-wheat group with that of rust resistance.

It was found, moreover, that in regard to other qualities than rust resistance it is not possible to obtain varieties which even approximate perfection, and especially is it rarely, if ever, true that many desirable qualities are found in the same variety. However rust resistant a certain variety may be, it will usually be found lacking in some other essential quality, and manifestly the most perfect rust resistance is of no consequence if other essential qualities are absent. As a rule the wheats that are most highly resistant to orange leaf rust[2] are not varieties of the common bread-wheat group (*Triticum vulgare*) at all, though it by no means follows that they can not be used in bread making. At the same time some of the most valuable sorts for bread flour, including a number of Russian varieties, rust very badly in certain seasons. Occasionally good qualities may neutralize bad ones in the same variety. For example, a variety may be very susceptible to rust when attacked, but usually be able to escape it by virtue of its quality of early maturity.

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[1] Cereal Rusts of the United States, Bul. No. 16, Division of Vegetable Physiology and Pathology, U. S. Department of Agr., 1899, by M. A. Carleton.

[2] For descriptions of the two wheat rusts of this country and illustrations of their differences see Bul. No. 16 of this Division, above referred to.

Consideration of such facts finally led to the determination to study thoroughly wheat varieties themselves in all their relations, and not simply wheat diseases. Such a study of course naturally presupposes the investigation of all associated problems, such as drought resistance, early maturity, yielding power, and other matters of great economic interest. The different phases of the subject of wheat culture in its broadest sense are so intimately connected that no one of them can be intelligently studied separate and apart from the others.

During the first season (1895) of the investigations above mentioned about one hundred crosses were attempted with wheat varieties (besides a number with varieties of oats), mainly to determine the facility with which hybrids might be produced by crossing varieties of quite different groups. One-third of these crosses resulted successfully, unless a few of them may possibly have resulted from accidental pollination. Some of them were readily effected between varieties of common wheat (*Triticum vulgare*) and the durums (*T. durum*), as well as between varieties of each of these groups and the poulards (*T. turgidum*). All the resulting hybrids were planted, but, the weather conditions of the following season being unusually severe, these and many of the other experimental varieties failed to survive.

This work was continued, but in the meantime careful studies were being made in the several wheat districts with a view of determining the particular needs of each. In some districts greater hardiness of winter sorts is required; in others, varieties with a particularly tenacious chaff; in others, stiffer straw; in others, drought resistance, and so on. Varieties bred for North Dakota and Minnesota are of no value for California, and the best varieties for Texas would be useless in Montana. But aside from these considerations a knowledge of the different botanical groups of wheats is necessary, in order to have at command all the sources from which may be drawn the qualities required for different districts.

After five years investigations it can by no means be assumed that a full knowledge of the conditions of wheat culture and the demands of the country has been attained by this Division. Nevertheless, it is now possible to establish a reasonably complete basis upon which intelligent and systematic work may be accomplished—work that either could not be accomplished at all from a narrower standpoint, or would require much more additional time than has been given to the acquirement of this foundation, and could not even then be as thoroughly done.

PERSONAL EXPLORATIONS.

At various times during the years 1894 to 1897 all the wheat States except New York, Pennsylvania, and the Pacific Coast States were pretty thoroughly explored by the writer, the conditions of soil and climate being noted and a careful study made of the nature and distribution of the wheat varieties. Finally, during the past season (1899), it became possible to make a similar investigation of such conditions in the Pacific coast and North Mountain States, special attention being given in this case to the region usually known as the Palouse Country, and also to wheat culture under irrigation. Naturally very valuable information was obtained through these personal observations, which will be of great use in future work in wheat improvement.

During the summer and autumn of 1898, under the direction of the Section of Seed and Plant Introduction of this Department, an exploration was made of the greater part of European Russia, including the Caucasus, and of a small portion of the Kirghiz Steppes, as well as of Hungary and Roumania, in search of additional cereals for this country. A general report of this work has been published.[1] In Hungary and Roumania no varieties better than our own were found that had not already been obtained from those countries. In Russia some very valuable sorts were secured, which together with four or five others yet to be received,[2] give this country now practically everything of importance in the line of wheats from that, the second greatest wheat country of the world. All these explorations have been of great value in furnishing a long-desired opportunity for a comparative study of wheat varieties and the conditions of wheat environment in different countries.

CHARACTERISTICS AND NEEDS OF THE SEVERAL WHEAT DISTRICTS OF THE UNITED STATES.

From the standpoint of investigations so far made concerning the conditions of wheat environment and the adaptations of varieties in the United States, the country may be considered as divided into eight wheat districts, each possessing characteristics quite different from those of the others. In fact, in some cases they are as different from each other as though they lay in different continents. They are as follows: (1) The Soft Wheat district, including mainly the New England and Middle States; (2) the Semihard Winter Wheat district, including the North Central States; (3) the Southern Wheat district, including the northern part of the Southern States; (4) the Hard Spring Wheat district, including the Northern States of the Plains;

---

[1] Russian Cereals adapted for Cultivation in the United States, Bul. No. 23, Division of Botany, U. S. Department of Agriculture, 1900, by M. A. Carleton.

[2] Since this was written these varieties have all been obtained.

(5) the Hard Winter Wheat district, including the Middle States of the Plains; (6) the Durum Wheat district, including a part of the Southern States of the Plains; (7) the Irrigated Wheat district, including in general the scattered portions of wheat area in the Rocky Mountain and Basin States; and (8) the White Wheat district, including the larger part of the Pacific Coast States. Just as these districts differ from each other in their characteristics, so do the particular needs of the wheat grower in each differ widely from those of other districts. (See colored map, frontispiece of this bulletin.[1])

GENERAL NEEDS OF ALL THE DISTRICTS.

Before describing these districts separately, it will be well to note briefly two general needs common to all of them. These are (1) greater yielding power and (2) earlier maturity. In the writer's experience these are found to be ever present needs, not only in all our own States but in all wheat countries.

YIELDING POWER.

This quality is of course always desirable, simply from the standpoint of obtaining the greatest possible profit from the same area. Nevertheless, on account of peculiar local conditions the demand for a large yield is given much more emphasis in some localities than in others. Besides, the need of a large yield does not always arise from the same cause, and in many cases it is not real, but only appears so because of defects in other regards. To illustrate, the Palouse country of Washington and Idaho may be taken as an example in contrast with that of the Southern States. In the Palouse country the regular average yield is already probably near 25 bushels per acre, while 35 or 40 bushels per acre is a common crop in certain seasons, and 60 bushels not particularly rare. Yet from no part of the country has the writer had more requests for information concerning larger-yielding varieties. As a matter of fact prices of wheat are proportionally so low on account of the great distance from good markets, and the method of summer fallowing, which allows a crop only every second year, is so much practiced that to overcome losses in these directions exceedingly large yields are considered necessary in order that much profit may be gained in the end. On the other hand, in the Southern States the problem of increasing the yield is entirely independent of deficiencies in other regards, for the home demand alone is sufficient to make prices good as a rule; but the average yield is extremely low, being under 10 bushels per acre. It would add one-half to the profit in these States if the yield could be increased even to the average of the entire country (slightly over 13 bushels per acre). In the South manuring the land must also be practiced in order to obtain the best results, which is an item not at present considered in the West.

---

[1] It has been a most difficult matter to prepare this map, and it is not claimed that it is accurate. Indeed it would be impossible at present to prepare an accurate map of this nature. But it represents approximately the different wheat districts characterized mainly by the cultivation of certain natural groups of wheats. Of course the different groups will lap over more or less from one district to another. In all that part of the United States approximately east of the one hundred and fourth meridian the uncolored portions represent territory either from which we have no statistics, such as the Indian Territory, or in which the wheat production averages less than 1 bushel to the square mile. West of this line the white portion represents territory in which there is practically no wheat grown at all. The reports of the census of 1890 and those of the Irrigation Division of the Geological Survey have been of much help in the preparation of the map.

In the States of the Plains the actual average yield is also rather low (a little over 12 bushels), so that here, too, the reason for a demand for an increased yield is evident and is usually independent of other deficiencies.

The average yield for the United States is far lower than it ought to be. The yield for the semiarid districts, which is much less, can and should be as high as that for the entire country at present.

EARLY MATURITY.

There is no part of the United States where early maturing wheats are not desirable for one reason or another. The reasons are various in different localities. As before stated, early ripening varieties are, in most seasons, more likely to escape damage by rust. In a large portion of the country this is a very important matter for consideration, but especially so in the Southern States and the States east of the Mississippi River, where the whole wheat crop is occasionally entirely destroyed by this parasite. But the need of early maturity is most urgent in the Palouse country, as the shriveling effects of the annual drought in that region which sets in just before harvest may be avoided by the use of early varieties. In the North Central States and the Great Plains region early maturing and winter varieties are less liable to the ravages of chinch bugs than are late maturing and spring varieties. In all the Northern States early maturity also allows the variety a better chance to escape early autumn frosts.

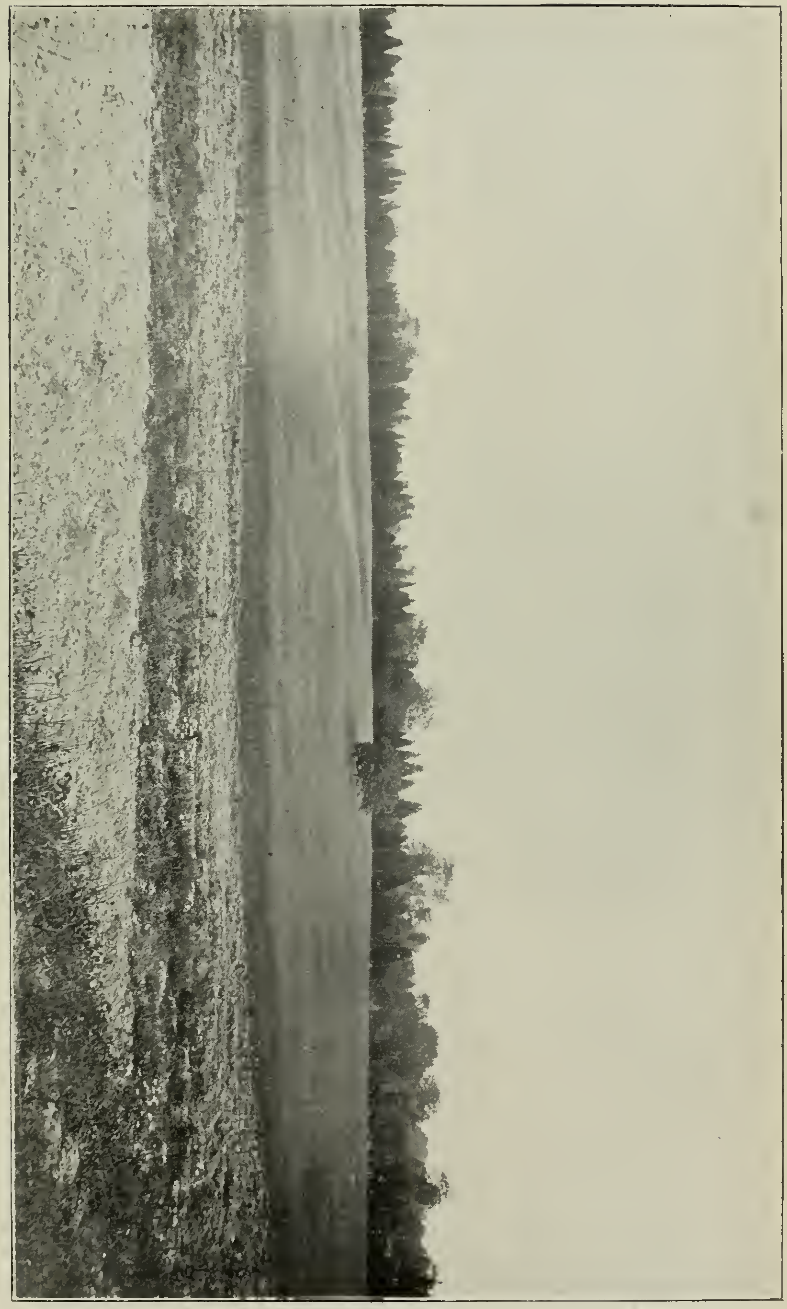
There are instances in which late maturity is apparently an advantage, but such cases are rare.

Finally it should be noted that there is quite a distinction between early wheats and early-sown wheats. A late-maturing wheat will ripen earlier than usual if sown earlier, or will ripen still later than usual if sown later. In the case of winter wheats early seeding allows the wheat plant to accumulate more reserve force in the roots during the autumn, thus enabling it to begin growth with greater vigor in the spring and get the start of the later-sown crops. In the case of spring sorts earlier seeding, of course, simply enables the crop to get an earlier start and thereby to ripen earlier. By early sowing and the constant selection of the earliest ripening heads for seed a naturally late wheat may be gradually transformed into an early variety.

SOFT WHEAT DISTRICT.

In this district are included approximately New York, Pennsylvania, New Jersey, Maryland, Delaware, and portions of Virginia (Plate I), West Virginia, and eastern Kentucky; also such portions of New England as produce wheat to any considerable extent. The region is characterized on the whole by the production of rather soft wheats, containing a large amount proportionally of starch, though occasionally they incline to semihard. The color of the grain is usually yellowish white or amber, but sometimes quite reddish. The soil, especially if not heavily fertilized, does not possess the necessary amount of alkali, phosphate, and humified organic matter required for the production of hard, glutinous wheats. Moreover the climate is against their production, being too moist and cool in summer. Nevertheless in New York and Pennsylvania, by means of the plentiful application of fertilizers and the unusual attention paid to seed selection practiced in this region, a large amount of good wheat is annually grown in proportion to the entire area. Twenty-five or thirty years ago, when the area given to wheat culture in this country was much more limited than at present, and when the hard red wheats were not so popular, New York had a deservedly great reputation both for her wheat production and flour industry. And even at present, if there is a diminution of this reputation, it is not because of any actual decrease in wheat and flour production, but because of the overshadowing increase in districts more favorably conditioned or situated, though we should add to this the fact that there has been a corresponding change in the kind of wheat used for bread making. The fact that so high a standard is maintained in the wheats of this region in the face of adverse natural conditions, is strong proof of the importance of intelligent wheat culture, particularly in respect to seed selection and the proper treatment of the soil. In some localities of this district the standard is considerably above what one would expect, while in some other districts it is far below what it should be.

In the most northern portions of this district spring sowing is almost entirely practiced, and there is a need for hardy winter sorts which will be able to extend the winter-wheat area farther northward. In some localities rust is occasionally very injurious, the black stem rust sometimes completely destroying the crop. Early maturing and rust resistant sorts are therefore desirable for escaping or overcoming the attacks of this parasite.



WHEAT FIELD NEAR MOUNT VERNON, VA. (ORIGINAL.)

SUMMARY OF CONDITIONS AND NEEDS OF THE DISTRICT.

(1) Chief varieties now grown:
    Fultz,
    Early Genesee Giant,
    Jones's Winter Fife,
    Red Wonder,
    Gold Coin,
    Fulcaster,
    Longberry,
    Mediterranean,
    Early Red Clawson,
    Blue Stem.
(2) Average yield per acre, about 14$\frac{2}{3}$ bushels.[1]
(3) Needs of the grower:
    (*a*) Harder-grained, more glutinous varieties.
    (*b*) Hardier winter varieties for the most northern portions.
    (*c*) Early maturity.
    (*d*) Rust resistance.

SEMIHARD WINTER WHEAT DISTRICT.

In this district we may include Ohio, Indiana, Illinois, Michigan, and a small part of Wisconsin. It produces a wheat of medium quality, and on the whole is one of the most important cereal regions of the United States. The wheats grown are generally semihard, rather reddish in color, and either bald or bearded. Throughout this district, as well as over a large portion of the country, there has been a decided tendency during the last twenty years or more toward the use of harder red wheats and also of a larger proportion of winter compared with spring varieties. The increasing use of the harder wheats has been coincident with the advent of the roller-milling process, but not necessarily a forced result of the latter, as some have inferred. The two have worked together. The proportion of such wheats now grown in this region is much larger than ten years ago. Especially is this true in Michigan, where special impetus has been given to such improvements through the efforts of Prof. R. C. Kedzie, assisted by the millers of the State. Similarly the area in which it is considered possible to grow winter wheats has been extended much farther northward, now including practically all of Michigan, nearly all of Illinois, and even a small portion of Wisconsin. Thus this group of States may now be properly called the semihard winter wheat district. These changes have been accomplished by the gradual introduction of hardier winter sorts, which are at the same time usually harder and red grained. Nevertheless there has been little more than a beginning in these improvements, and there is still a demand for hard red wheats, and in the northern portion of the region for hardier winter varieties.

The black stem rust is sometimes very destructive in these States, particularly in the lower, moist, and timbered portions of Ohio, Indiana, and Michigan. Hence there is great demand also for rust resistant sorts.

---

[1] Calculated as accurately as possible from data collected by the Division of Statistics of this Department covering the period 1890–1899.

SUMMARY OF CONDITIONS AND NEEDS OF THE DISTRICT.

(1) Chief varieties now grown:

Fultz,	Poole,
Rudy,	Valley,
Early Red Clawson,	Nigger,
Dawson's Golden Chaff.	

(2) Present average yield per acre, about 14 bushels.

(3) Present needs of the district:

  (*a*) Hardness of grain.
  (*b*) Rust resistance.
  (*c*) Hardy winter varieties.

SOUTHERN WHEAT DISTRICT.

In area this district includes the larger portion of Kentucky, Virginia, West Virginia, and North Carolina, all of Tennessee, and portions of South Carolina, Georgia, Alabama, Arkansas, and Missouri. The annual production of wheat is comparatively small, and is furnished principally by Kentucky, Missouri, Tennessee, and Virginia. In the greater portion of the region the combination of great rainfall with mild temperature is not conducive to the greatest success in wheat growing. The soil is also generally not of the best for such purposes. Rust is always very bad, because of the constantly damp, warm climate. In spite of these difficulties there is no doubt that with sufficient effort the wheat industry might be very materially improved. Just recently there has been much interest awakened in the possibilities of successful wheat culture, particularly in Georgia and South Carolina. This increasing interest in the matter finally resulted in the calling together of a convention at Macon, Ga., in July, 1899, when it was unanimously decided that Georgia can very easily and should supply her own demands for wheat for bread making. Many members of the convention gave very favorable testimony regarding their own experiences in wheat growing during the past year. Probably one of the greatest obstacles in the way of profitable wheat raising in portions of the South is the lack of good flouring mills, much of the grinding being at present performed by the most primitive of gristmills. With a continued increase in wheat acreage there will perhaps be a corresponding increase in the number of first-class mills constructed.

On account of the severe rust attacks which occur in this district it is highly desirable to grow early ripening and rust resistant sorts. But there are really not many early maturing wheats grown in this country, and of the early foreign varieties already tested none have yet proved to be sufficiently hardy. Canning Downs, an early Australian sort, winterkilled even in so mild a region as Mississippi.[1] However, there has not been a sufficient number of trials of such varieties, and the different experiments have not been often enough repeated to give reliable results. As to the matter of rust resistance, experiments made in Louisiana[1] showed that hard red wheats, including a number of Russian origin, resisted rust the best. In Mississippi two Australian varieties, Beloturka and Defiance, were quite rust resistant, while varieties obtained from England rusted very badly.[2]

---

[1] See Tracy, S. M. Wheat. Sixth Annual Report Mississippi Agricultural Experiment Station, 1893, pp. 23–25; also Eighth Annual Report, 1895, pp. 44–46.

Occasionally wheat is much injured in the northern portion of this region by late spring frosts. It is on such occasions that late-maturing wheats and late-sown crops may have the advantage, since those ripening early are likely to be caught by the frost just at blooming time and be prevented from "filling out," while the later ripening crops, blooming after the frost, escape such injury. It seems possible, however, to grow varieties that will resist the action of these frosts, and therefore varieties hardy in this respect are desirable.

The wheats at present grown in the Southern Wheat district are either soft or semihard, and usually amber or reddish in color. They are either bearded, as in the case of the Fulcaster, or beardless, of which the Fultz and May wheats are examples. In Arkansas and the Carolinas, Nicaragua wheat, a durum variety, is grown somewhat, but to no great extent as yet. Wheat from the Southern States is always more likely to be infested with weevil than that from other districts, and occasionally much annoyance as well as injury to the grain results from this cause. Nicaragua and the hard red wheats are more resistant to weevil than are the soft wheats.

SUMMARY OF CONDITIONS AND NEEDS OF DISTRICT.

(1) Principal varieties at present grown:
    Fultz,
    Fulcaster,
    Red May,
    Currell's Prolific,
    Rice,
    Everett's High Grade,
    Boughton,
    Purple Straw.
(2) Present average yield per acre, about 9⅔ bushels.
(3) Needs of the grower:
    (*a*) Rust resistance.
    (*b*) Early maturity.
    (*c*) Resistance to late spring frosts.
    (*d*) Stiffness of straw.

HARD SPRING WHEAT DISTRICT.

The hard spring wheat area comprises the States of Minnesota, North Dakota, South Dakota, the larger part of Wisconsin, portions of Iowa and Nebraska, and small portions of Montana and Colorado.

---

[1] See Stubbs, W. C. Experiments in wheat. Louisiana Agricultural Experiment Station Bulletin No. 19, 1892, 2d series, pp. 555–562.

[2] See Tracy, S. M., in Mississippi Agricultural Experiment Station reports above cited.

In this district, because of the rich, black soil and dry, hot summers, there is grown the highest grade of spring wheat in the world, excepting the spring varieties of the middle Volga region in Russia, which are very similar.

Two general types of wheat prevail throughout this district—the Velvet Blue Stem[1] and the Fife. A large proportion of the farmers in this region know no wheat which does not belong to one of these types. The chaff of the Velvet Blue Stem is covered rather closely with small hairs, and the plants are bluish gray near harvest time. In both types the heads are beardless and the grains are medium or small, hard, and red. There are several strains or varieties of each type. The gluten content of these wheats is comparatively very large, and especially of that quality which gives great lightness in bread making.

The average annual wheat production of this district is larger than that of any other similar area in the world, and is about 30 per cent of the entire production of the United States. The average yield per acre, however, is not very large—certainly far below what it might be. Almost everywhere the self-binder is used in harvesting the grain, and in some localities the farms given entirely to wheat culture cover many thousand acres. (See Plate II.) On these bonanza farms 50 to 100 self-binding harvesters are sometimes at work at the same time. The large size of the farms is one of the worst features connected with wheat growing in the Northwest. From this cause not enough attention is given to details of the work. Operations delegated to the best of foremen and other employees are never so carefully performed as when done under the direct scrutiny of the man who owns the farm, and whose interests are therefore at stake. Little things that are of importance when summed up are overlooked. The tillage is not thoroughly accomplished, weeds are not kept down, there is more or less waste of land, and the grain is allowed to degenerate in quality.

The needs of the grower in this district are not so great as in some others, though there is much to be desired. In the northern portion earliness of maturity is needed to enable the wheat to escape the early autumn frosts which sometimes catch the crop before harvest, while in the southern portion chinch-bug depredations and rust attacks might often be avoided through possession of the same quality. A combination of earliness and rust resistance in the same variety would be especially desirable. The average yield could be made very much larger, as already stated, but this is a matter depending fully as much on methods of culture as on the improvement of varieties. Proper seed selection,

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[1] There are apparently four distinct varieties of so-called Blue Stem in the United States. The name Velvet Blue Stem is adopted here to designate the spring variety grown in this district. The one grown in the Palouse country will be called Palouse Blue Stem.

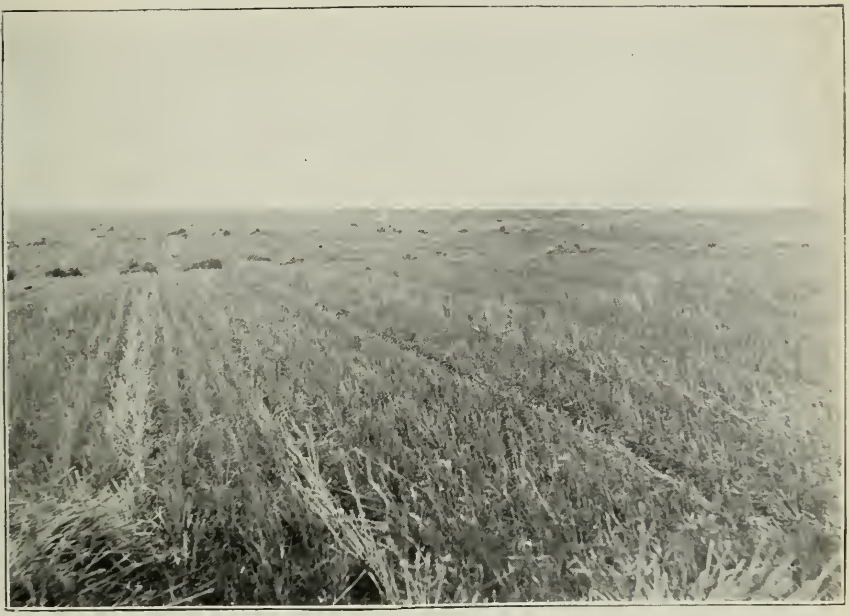

FIG. 1.—WHEAT FIELDS OF THE RED RIVER VALLEY, NEAR GRAND FORKS, N. DAK. (ORIGINAL.)

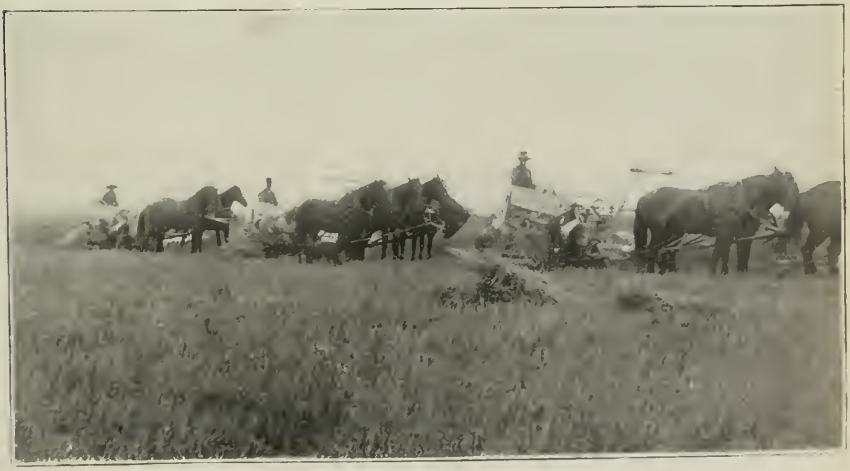

FIG. 2.—SELF-BINDERS AT WORK NEAR GRAND FORKS, N. DAK. (ORIGINAL.)

however, should be rigidly practiced. The establishment of hardy winter varieties in place of the spring varieties now grown would no doubt be an improvement of the utmost value in Iowa, Nebraska, and portions of Wisconsin, and perhaps a small part of Minnesota. This border is now the battle ground between winter and spring varieties, and it should be the constant aim to carry the line farther to the north, thus increasing more and more the winter-wheat area. Such purpose can be accomplished either (1) by the introduction of winter varieties, of similar quality to the spring sorts now grown, from the Crimea, north Caucasus, and southern Volga region of Russia, or (2) by the actual origination of hardier winter varieties of good quality through hybridization and selection. As an example of the effectiveness of the former method, we have only to point out the work already accomplished by Turkey wheat—a Crimean variety—in extending the winter-wheat area in Nebraska and Iowa.

SUMMARY OF CONDITIONS AND NEEDS OF THE DISTRICT.

(1) Principal varieties at present grown:

Saskatchewan Fife,	Hayne's Blue Stem,
Scotch Fife,	Bolton's Blue Stem,
Powers Fife,	Wellman's Fife.

(2) Average yield per acre, about 13 bushels.
(3) Needs of the grower:
  (*a*) Early maturity.
  (*b*) Rust resistance.
  (*c*) Hardy winter varieties.
  (*d*) Drought resistance.

HARD WINTER WHEAT DISTRICT.

In this district is comprised approximately the middle States of the plains, including Kansas, a large part of Missouri, portions of Iowa and Nebraska, and the larger part of Oklahoma. As the name implies, it is characterized by the production of hard winter wheats, such wheats as are rarely found, but which are of the very best quality. The only other wheat region in all the world that is exactly comparable to this one, so far as known, is that including northern Crimea and the country directly between the Sea of Azov and the Caspian Sea. The latter region, however, at present produces better wheats than are produced in this district, and therefore should be drawn upon for all improvements that are attempted through introduced sorts.

The wheats of this district have slender, stiff stems, narrow compact heads, usually bearded, and medium or small, hard, red grains. In this region there is the most interesting example of the changes that may take place for the better in the development of the wheat industry. Twenty-five years ago the softer wheats (often white-grained) were chiefly grown over a large portion of this district, and the cases of winter wheat sowing as against spring wheat sowing were much fewer than at present. Now the hard red-grained varieties are principally used, and only in Iowa and Nebraska are spring varieties grown to any extent. The introduction of these hard-grained winter sorts has added remarkably to the certainty and value of the wheat crop, and has greatly decreased the ravages from rust and chinch bugs.

Such improvements are after all but fairly begun, and there is yet great demand for hard-grained sorts and varieties that will resist the winters of Iowa and Nebraska. As the wheat area extends farther westward—to the one hundredth meridian and beyond—there is also a special need for drought-resistant sorts. In fact, in this and the district just described there is the most exacting demand of the entire country for hardy varieties. The extreme severity of the drought and winter cold combined forms a greater obstacle to winter wheat culture than exists in any other district. The average yield per acre is always low, but the problem in a considerable portion of the region is not so much to increase the yielding power per acre as to make sure of a crop every year, since there are so many complete failures from drought. A constant average of even 12 to 15 bushels per acre from year to year would be considered good.[1]

Early maturity is of importance in this district in order to allow an escape from the worst effects of the drought in the western portion and from the rust in the eastern portion. Rust resistance is also important, but not so much so as in States east of the Mississippi River.

SUMMARY OF CONDITIONS AND NEEDS OF THE DISTRICT.

(1) Chief varieties at present grown:
    Turkey,
    Fulcaster,
    May,
    Zimmerman,
    Fultz.
(2) Average yield per acre, about $12\frac{2}{3}$ bushels.
(3) Needs of the grower:
    (*a*) Hardy winter varieties.
    (*b*) Drought resistance.
    (*c*) Early maturity.

DURUM WHEAT DISTRICT.

The area contained in this district is comparatively small and includes a large part of north-central Texas, the southwestern portion of Oklahoma, and a small portion of the southwest corner of Kansas. It also properly includes a portion of Colorado, but can not be so indicated on the map, as the particular portion is not yet definitely outlined. Some of this region (southwestern Oklahoma) has only recently been opened to settlement, but wheat culture has developed rapidly in the new lands. The soil is generally black and rich in humus, just as in the district last described, and produces wheats with a large gluten content, which quality is further increased in the western portion by the dry, hot summer weather. The general demand is for hard-grained, drought-resistant varieties, and such sorts are already grown to a considerable extent. In recent years there has been an increasing tendency toward the cultivation of the durum or macaroni wheats, the chief variety grown so far being Nicaragua which has become quite popular. This variety is very hardy, yields well, and the grain is extremely hard and glutinous. It is quite similar to Kubanka, Arnautka, and other macaroni wheats grown in southern Russia, and for which there is so much demand in France and Italy. Notwithstanding the usual notion concerning such wheats, Nicaragua has been very successfully ground into flour by a well-known milling company at Fort Worth, Tex. By mixing slightly with other wheats an excellent bread flour is made. However, the chief profit to be gained from the cultivation of this variety in future will no doubt arise from its use in manufacturing macaroni, just so soon as the possibility of furnishing a sufficient supply becomes certain. Though its distribution is not yet very wide, Nicaragua is, nevertheless, grown over a large portion of Texas and also sparingly in Oklahoma and Colorado. For this reason, and because of the evident adaptation of such wheats to this region, it seems proper to call it the durum wheat district.

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[1] The problem of successful wheat growing in arid regions is receiving earnest consideration and will be discussed in a later publication.

These durum wheats grow rapidly, are tall, and have wide leaves with a harsh surface, and large heavy-bearded heads, compactly formed. The grains are very large and long, and yellowish-white in color, becoming darker the blacker the soil in which the crop is grown. It being once proved that durum wheats succeed well, there is bound to be a still greater demand for them, so that the further introduction of such varieties becomes at once one of the needs of the district. Aside from macaroni varieties, the red-grained winter wheats, similar to those described for the Hard Winter Wheat district, are best adapted for the larger part of this region. The best example is the Mediterranean, which is very commonly grown.

In central and southwestern Texas rust is very destructive, so much so that wheat culture has been completely abandoned in many places on account of it. There is, therefore, a great demand for rust resistant varieties. The durum wheats have the advantage of being highly resistant to orange leaf rust, but succumb to black stem rust. In the western portion of the district the oft-recurring droughts are very detrimental, and therefore in that portion drought resistance and early maturity are important qualities.

SUMMARY OF CONDITIONS AND NEEDS OF THE DISTRICT.

(1) Chief varieties at present grown:

Mediterranean,	Fulcaster,
Nicaragua,	Turkey.

(2) Average yield per acre, 11½ bushels.

(3) Needs of the grower:
  - (*a*) Macaroni varieties.
  - (*b*) Drought resistance.
  - (*c*) Rust resistance.
  - (*d*) Early maturity.

IRRIGATED WHEAT DISTRICT.

In this region is included all those scattered portions of the Rocky Mountain and Basin States in which wheat is grown at all. The States thus included are Wyoming, a part of Montana, southern Idaho, Utah, Nevada, Arizona, New Mexico, and the greater part of Colorado. In this district we find conditions remarkably different from those existing anywhere east of the Rocky Mountains. Three striking characteristics not present to so great a degree in any other district are (1) the extreme aridity, necessitating the application of water by irrigation, (2) the very low humus content of the soil, and (3) the superabundance of alkali usually present. These conditions are closely interrelated and mutually dependent upon one another. The absence of humus is a natural result of the absence of rainfall, upon which depends the existence of plant life. Rainfall also tends to equalize the distribution of the alkaline matters of the soil, which in this district, however, are concentrated, in places, in high percentages. The practice of irrigation is often allowed to make conditions worse by gradually carrying and depositing in certain localities or on certain farms an excess of alkali largely above that which was already present. These features of extreme aridity, lack of humus, and excess of alkali are so particularly characteristic that they go far beyond any matters of temperature dependent upon latitude or elevation in their effects upon the nature of wheat varieties grown in this district. That is, wheats so far north as southern Idaho are very like those of southern New Mexico or Arizona, and in all parts of the district show uniformly a great lack of gluten content, which is dependent mainly upon the presence of soil humus.

Wheat does best in soil that is alkaline rather than acid in reaction, but an excess of alkali becomes very injurious. Different cereals are able to withstand different amounts proportionally of alkali in the soil. Barley and rye seem to tolerate a larger proportion than wheat, and the latter will usually tolerate a larger amount than oats. Of all the cereals barley will withstand the largest amount.

The wheats of this district are almost always white-grained, soft, and extremely starchy, and lack greatly in gluten content. The straw is so white and clean and glistening that it is dazzling to the eyes in the hot sunshine. Rust on wheat is seldom injurious, and in some localities is entirely unknown. Smut, however, is often present to a considerable extent. The stiffness of the straw and the absence of rain prevent the grain from ever lodging, so that harvesting may be delayed for weeks with little or no injury to the grain.

Manifestly the greatest need of this district is an increase in the gluten content of the grain. While the introduction of hard-grained nitrogenous sorts from other sections is at first an improvement, the gluten content can not thus be materially and permanently increased. No wheat variety, whatever its nature, can abstract from the soil elements that are not present there. Wheats brought from the black prairie soils of other sections to this district show the most striking illustration of the radical changes that may be caused in a variety by a simple transference to a new locality, and, even when grown under the best of care, quite effectually disprove a notion prevalent even among scientists that varieties will not deteriorate. The hardest red Fifes from North Dakota, Turkey wheat from Kansas, or Diamond Grit from New York become rapidly more starchy and of a lighter color on being grown in Utah or New Mexico. The first requisite, therefore, for wheat improvement in irrigated sections is the complete amelioration of the soil by (1) dispersing the excessive accumulations of alkali and (2) increasing the humus content through the application of nitrogenous fertilizers and the growth of leguminous crops in alternation with wheat. At the same time it will aid greatly to gradually introduce the harder red-grained wheats.

In many portions of this district, at high elevations in the mountains, wheat is often seriously damaged by early autumn frosts. It is therefore important to obtain for these localities the earliest maturing varieties possible, or varieties that may perhaps resist the action of the frost. For example, in the San Luis Valley of Colorado wheat is grown at an elevation of over 7,500 feet, where frost is likely to occur in any month of the year, but is especially liable to injure the crop in August.

SUMMARY OF CONDITIONS AND NEEDS OF THE DISTRICT.

(1) Chief varieties now grown:

Sonora,	Little Club,
Taos,	Defiance,
Felspar,	Amethyst.

(2) Average yield per acre, about 21 bushels.

(3) Needs of the grower:
  (*a*) Increase of the gluten content.
  (*b*) Early maturity.

WHITE WHEAT DISTRICT.

This district covers, in a general way, the Pacific Coast region, including California, Oregon, Washington, and northern Idaho. All varieties that have become at all acclimated are characteristically white-grained, soft, and starchy. Usually the factor which is probably most influential in producing a grain of such nature is the lack of humus in the soil, as is true in the irrigated district. The generally cool summers, however, no doubt give aid to the same end. Hard red-grained varieties, when brought to this district, deteriorate in a few years time. Nevertheless such introductions have in a number of instances proved beneficial.

A majority of the more common varieties strictly characteristic of the district are of the group usually called club wheats and belong to the species *Triticum compactum*. Sonora, Defiance, and Australian of California, Red Chaff of Oregon (distinct from the Palouse Red Chaff of the Palouse country), and Palouse Blue Stem of Washington are not, however, club wheats. As the botanical name of the club group implies, these wheats have their spikelets (meshes) so compactly arranged in the heads that they stand out nearly at right angles with the rachis (or stem of the head). The head thus becomes squarely formed (hence the name square head applied to many of the varieties), and, being usually a little larger at the apex than at the base, appears club shaped. Thus, although the heads are usually rather short, each contains comparatively a large number of grains, which partially accounts, probably, for the large yields per acre in this district. Heads of Chili Club are occasionally found that contain over 160 grains each.

A very valuable characteristic of the club wheats is their ability to hold the grain in the chaff so that there is little danger of shattering, even during the driest season, if there should be much delay in the harvest. In some localities the grain, though ripening in July, is sometimes left standing till September before harvesting, a habit which, however, has no good excuse for its practice.

For the purpose of clearer discussion, the district may be considered as subdivided into three sections—California, Oregon, and the Palouse country of Washington and northern Idaho.

In southern California the varieties Sonora and Defiance are much grown, the latter particularly for its rust resistance, which is an important need in this part of the State. Sonora wheat has a reddish velvet chaff, is beardless, and is white-grained as seen in this district. The grain is a little harder than that of the club wheats and is used for export, while the grain of the latter is used for home consumption.

From the latitude of Fresno to the Oregon State line Australian and the various strains of club wheats are principally cultivated. The best known varieties that are given special names at all are Golden Gate Club, Salt Lake Club, and Chili Club. The variety Propo is also

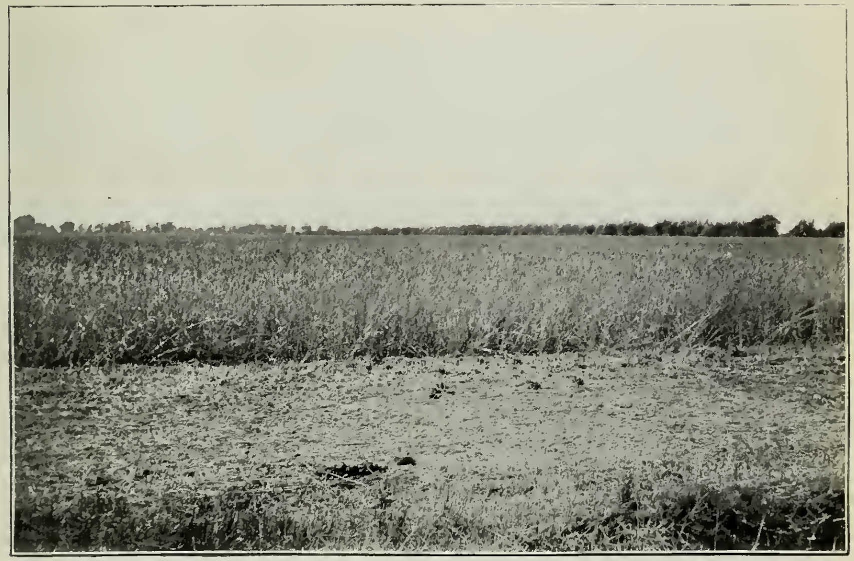

FIG. 1.—FIELD OF WHEAT ON "TULE" LANDS NEAR STOCKTON, CAL. (ORIGINAL.)

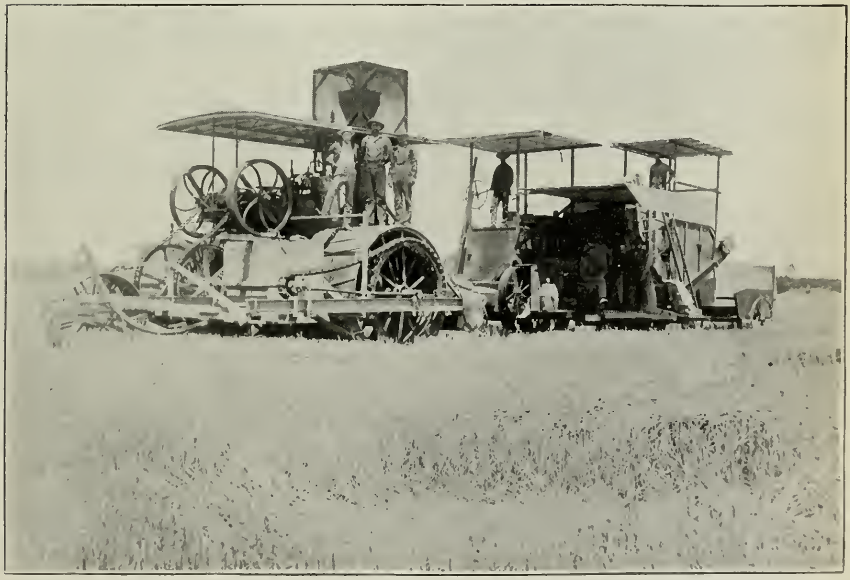

FIG. 2.—STEAM COMBINED HARVESTER-THRESHER HARVESTING ON "TULE" LANDS NEAR STOCKTON, CAL. (ORIGINAL.)

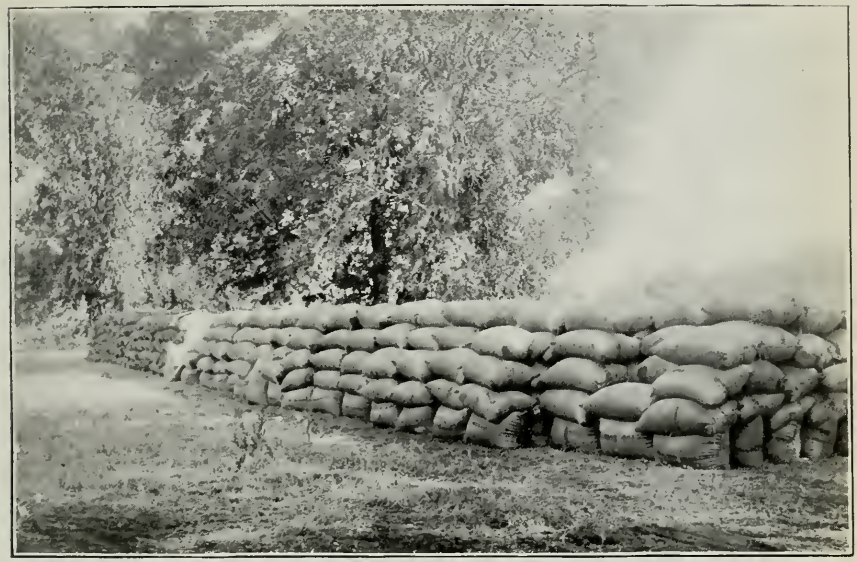

FIG. 8.—BAGS OF WHEAT JUST HARVESTED ON THE BIDWELL ESTATE, CHICO, CAL. (ORIGINAL.)

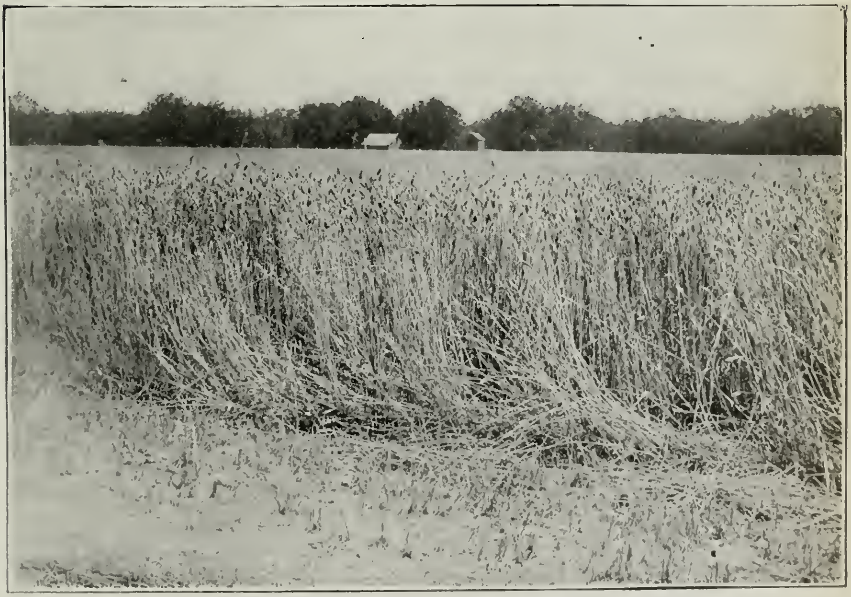

FIG. 2.—WHEAT FIELD NEAR TEHAMA, CAL. (ORIGINAL.)

grown to some extent. Other sorts from the East, such as Rudy, are occasionally introduced, but these do not seem to yield so well, and besides shatter so badly that they soon have to be given up. Nonshattering varieties are in great demand. In all portions of the State the increase of the gluten content is probably the greatest need. All varieties grown in the State are winter wheats.

One of the most interesting sections of California devoted to wheat culture is that of the "Tule" lands, near Stockton. (See Plate III, fig. 1.) The great grain fields there show strikingly the possibilities in a reclamation of immense marshes. They were once vast flats covered with water, mud, and a growth of bulrushes (*Scirpus lacustris*), called Tule in Spanish. By means of pumping, dredging, and throwing up levees these lands have been reclaimed, and now after many years they are among the most fertile of the State. Wheat yields from 50 to 80 bushels per acre here, and barley sometimes as much as a hundred bushels or more per acre. This remarkable fertility is a result, in part at least, of the deep deposits of organic matter. There is still apparently a lack of certain mineral ingredients, such as lime and potash, which are needed to make the quality of the grain as good as the quantity.

As in the case of the Hard Spring Wheat district the chief difficulty in the way of successful wheat culture in California, so far as agricultural practice is concerned, is the enormous size of many of the farms or ranches. They are even larger than in the Dakotas and Minnesota, containing often from 20,000 to 30,000 acres. On this account it is impossible to give the attention to details in farming that are necessary for the best results. The lack of attention to nitrogenous manuring, and especially to the alternation of wheat with leguminous crops, is particularly noticeable.

The combined harvester-thresher (Plate III, fig. 2) is used in harvesting pretty generally throughout the State. This machine is either drawn with an engine or with 28 to 40 horses. By its use the grain is thrashed directly from the field, and left piled in bags. (See Plate IV, fig. 1.) Immense ricks of these bags of grain remain in the field sometimes for weeks unmolested and undamaged by the weather. All grain throughout the State is handled in this form and calculations are made in bags and not in bushels. There is therefore no use for the grain elevator, in the ordinary sense of the term. Each bag contains 2½ bushels or about 150 pounds.

West of the Cascades, in Oregon, conditions are somewhat similar to those in California. In a large portion of the State a considerable amount of spring wheat is grown. In addition to the ordinary club wheats some other varieties, such as Oregon Red Chaff and Foise, are also well represented. The midsummer climate is much cooler than in California, and therefore harvesting is performed much later. On account of the greater dampness of the atmosphere and the smaller size of the farms combined harvester-threshers are not used, but self-binders instead. There is great need of early maturing varieties, as the cool autumn weather begins so early. The nitrogen content of the grain is exceedingly small.

In eastern Oregon climatic and other conditions are quite different from those west of the Cascades, and a description of that section is more properly included in the discussion of the Palouse country.

In western Washington the general conditions and the quality of the wheat are very similar to those of western Oregon, but in southeastern Washington and adjacent portions of Idaho and Oregon is a large section known as the Palouse country, which possesses peculiarities of soil and climate that are distinctively characteristic and radically different from those of the Pacific Coast region proper. Strictly speaking, the Palouse country is considered to be rather limited in extent, comprising approximately Latah County, Idaho, and Whitman County, and very small adjoining portions of Adams and Franklin counties, in Washington. Recently, however, the term has come to be applied practically to nearly all of these last-named counties, as well as to Garfield, Columbia, and Walla Walla counties (Plate V), and may even include the northern portion of Umatilla County, Oreg. The two features which most distinguish this region from the Pacific Coast proper are the dryness of the climate and very finely divided condition of the soil. The particles are so very fine that when dry the soil is practically mere dust. On windy days this dust fills the air, forming vast clouds that are very disagreeable to the traveler. At the same time, with very little rain the soil becomes quite sticky and difficult to manage. The capacity of the soil to absorb and retain moisture is remarkable. It is pretty generally believed that a rainfall of 12 inches in this district is sufficient to make a crop of wheat, while in the States of the Plains 18 inches is considered to be rather low for successful wheat growing. Wheat is the chief crop of the region, though barley and oats are grown to some extent. The principal wheat varieties (except Palouse Blue Stem) are of the club-wheat group. They are usually soft grained and starchy, and generally white, similar to those of the coast region, but a little better in quality. The three standard varieties commonly grown are Palouse Blue Stem, Palouse Red Chaff, and Little Club. As regards the comparative distribution of these varieties, if the region be considered as divided into three parallel north and south belts, it will be found that Palouse Blue Stem prevails in the western belt, extending as far westward as North Yakima; Palouse Red Chaff in the middle belt, passing through the heart of the region, and Little Club in the eastern belt, reaching the foothills of the mountains.

The most serious obstacle to successful wheat culture in the Palouse country is the annually recurring drought which occurs about two weeks before harvest time, particularly in the western and southern

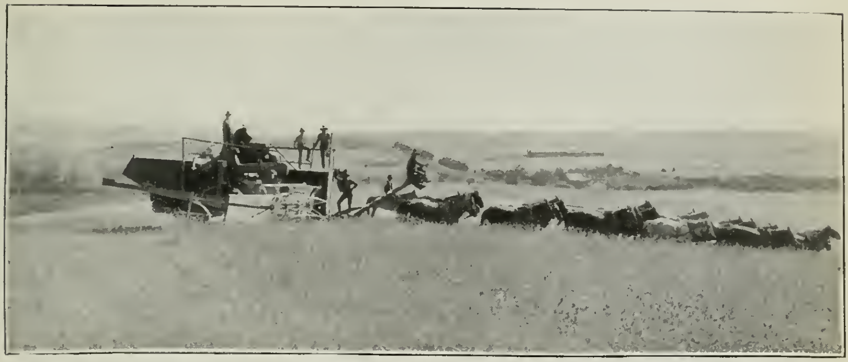

FIG. 1.—HARVESTING WITH THE COMBINED HARVESTER-THRESHER NEAR WALLA WALLA, WASH. (PHOTOGRAPHED BY A. B. LECKENBY.)

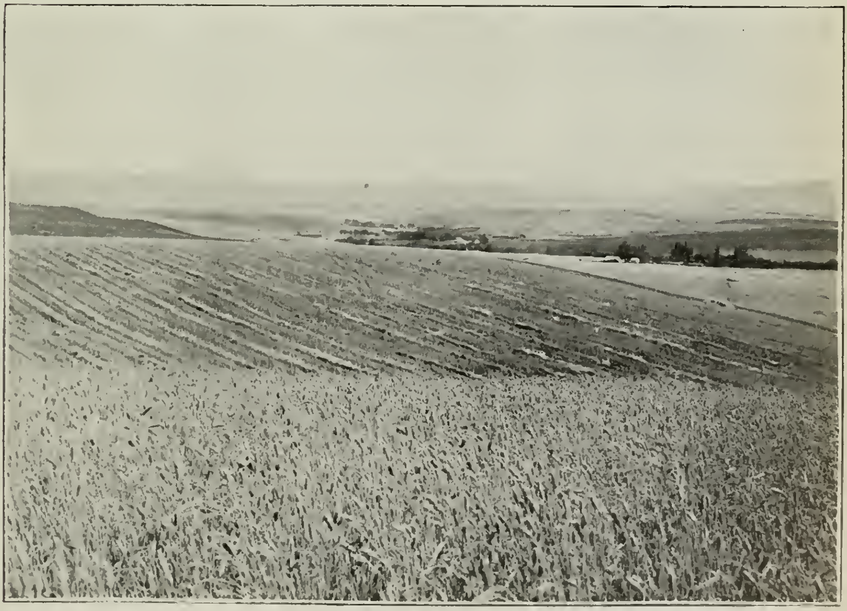

FIG. 2.—WHEAT FIELDS BEFORE AND AFTER HARVESTING, NEAR WALLA WALLA, WASH (PHOTOGRAPHED BY A. B. LECKENBY.)

portions. From this cause the wheat is often badly shriveled, and both the yield and quality thereby much affected. A slight compensation for this loss lies in the fact that shriveled wheat in this district is more in demand for making macaroni than plump wheat, because of the greater proportional amount of gluten in the former. In order to escape the severe effects of the drought, early maturing sorts are exceedingly desirable. It would probably be no exaggeration to say that a variety ripening ten to fifteen days earlier than the varieties now used, and as good in other respects, would add from one to three million dollars a year to the wealth of this region. In the central and southern portions of the region fall sowing is chiefly practiced, but in the northern and eastern portions, near the mountains, there is a larger proportion of spring varieties, and there a good, hardy winter sort is needed. In the drier western and southern portions, especially in the vicinity of Walla Walla, nonshattering varieties are necessary. There the combined harvester-thresher (Plate VI, fig. 1) is used in harvesting, as in California. In the north and east, and in the more hilly portions, as in the vicinity of Colfax, the self-binder is more commonly employed. In a few places a comparatively new sort of machine has recently come into use. (Plate VI, fig. 2.) It makes a 10 or 12 foot cut, and is driven in front of the horses, as in the case of a header, but unlike the latter possesses a self-binding attachment as well.

SUMMARY OF CONDITIONS AND NEEDS OF THE DISTRICT.

(1) Principal varieties at present grown:
    Australian,
    California Club,
    Sonora,
    Oregon Red Chaff,
    Palouse Blue Stem,
    Palouse Red Chaff,
    Little Club,
    White Winter,
    Foise.

(2) Average yield per acre, about 14⅞ bushels.

(3) Needs of the grower:
    (*a*) Early maturity.
    (*b*) Nonshattering varieties.
    (*c*) Hardy winter varieties in the colder portions.

SOURCES FOR DESIRABLE QUALITIES.

Having described the characteristic features of the different wheat districts of the country, and having noted the most pressing needs of the grower in each one, respectively, it will now be appropriate to discuss the sources from which the desirable qualities may be obtained for satisfying these needs. This subject may be considered from two different standpoints, (1) the botanical subdivisions of the cultivated varieties of wheat (*Triticum*) in the broadest sense, and (2) the geographic groups of varieties characteristic of different regions of the world. Manifestly a complete treatment of the subject can not be presented in the present state of our knowledge, since wheat varieties and their adaptations have not been thoroughly studied in all parts of the world. Nevertheless, considerable investigation has been made in this line, and the future promises still more. Such studies are exceedingly interesting, and form an absolutely necessary part of the basis for rational wheat improvement.

CHARACTERISTICS OF BOTANIC GROUPS OF WHEAT.

The cultivated varieties of *Triticum*, according to Körnicke and Werner,[1] whose classification will in the main be followed in this bulletin, may be grouped into eight species and subspecies, as follows: *Triticum vulgare*, *T. compactum*, *T. durum*, *T. turgidum*, *T. polonicum*, *T. spelta*, *T. dicoccum*, and *T. monococcum*. Only *T. vulgare*, *T. polonicum*, and *T. monococcum* are considered to be good species in all classifications. The other five are generally considered as subspecies of *T. vulgare*, though *T. compactum* is sometimes not even elevated to that rank. In this bulletin they will all be referred to as though they were distinct species. The chief characters of these groups of wheats will now be described, with special reference to their importance in wheat improvement.

COMMON BREAD WHEATS (*Triticum vulgare*).

This is of course the most valuable and widely distributed group of wheats in the world, and is represented by a greater number of varieties than all other species taken together. Nevertheless a number of very important qualities can be found only among varieties of the other species.

The characters of this group, both botanical and agricultural, are well known. The heads are long in proportion to thickness, as compared with those of some other groups. They are broader in the plane of the rows of spikelets, as a rule, and narrower on the sides of the furrow between the rows; taper toward the apex, but may be very blunt or even thicker above; are usually loosely formed comparatively, bearded or bald, and usually possess smooth chaff, but may be velvety. The spikelets, or meshes, as they are popularly called, generally contain three grains, but sometimes two and rarely four. The empty glumes or outer chaff of the spikelets are slightly keeled above and merely arched below. The stem of the plant is usually hollow, but occasionally somewhat pithy within and varies greatly in strength and height in different varieties. The leaves also vary in character, but are rarely as wide as those of the durum and poulard groups, and are velvety in only a few varieties.

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[1] Körnicke, Fr., und Werner, H. Handbuch des Getreidebaues, 1885.

The species is usually divided into a number of botanical subspecies and varieties, based upon the presence or absence of beards, nature and color of the chaff, color and quality of the grain, etc. For our present purpose, however, it will be more useful to consider that there are five great subdivisions of the species, based not upon botanical characters, but upon characteristics induced by influences of environment, as follows: (1) Soft Winter wheats, (2) Hard Winter wheats, (3) Hard Spring wheats, (4) White wheats, and (5) Early wheats.

The location of these groups in the United States has already been pretty well stated in the descriptions of our wheat districts. Their distribution throughout the world is approximately as follows: (1) The soft winter wheats, varying in color of grain from amber to white, are produced under the influences of considerable moisture and mild, even temperatures, and are distributed in the Eastern United States, western and northern Europe, Japan, and in portions of China, India, Australia, and Argentina. (2) The hard winter wheats are red-grained, usually bearded, possess a relatively high gluten content, and are more limited in their distribution. They are grown usually on black soils and under the influences of a climate characterized by extremes of temperature and moisture, but especially by dry, hot summers. They are found chiefly in the States of Kansas, Nebraska, Iowa, Missouri, and Oklahoma in this country, in Hungary and Roumania, in southern and southwestern Russia, and to some extent in northern India, Asiatic Turkey, and Persia. (3) The hard spring wheats are also red-grained and rich in gluten content, and are adapted to conditions of soil and climate identical with those just mentioned for hard winter wheats, with the exception that the growing season is shorter and the winters too severe for winter varieties. They are found in central and western Canada, our Northern States of the plains, east Russia, and western and southern Siberia. (4) The white wheats are soft and very starchy, but possess grains a little harder and much drier than those of the soft winter wheats. They are either fall or spring sown, and are sometimes sown at both seasons in the same locality. They are grown chiefly in the Pacific coast and Rocky Mountain States of this country, in Australia, and in Chile, Turkestan, and the Caucasus. (5) The early wheats are soft or semihard and generally amber to red in color of grain, but are distinguished from other groups chiefly in their ability to ripen early. They are found in Australia and India, are represented by a very few varieties in the Southern States of this country, and include some of the dwarf wheats of Japan.

The varieties of this species naturally include the most diverse characters, because of their cultivation under so many diverse conditions. Their greatest characteristic as a whole, however, is, of course, the well-known and long-established quality of their grain for the production of bread flour, for which reason the term "bread wheat" is usually applied to them. Nevertheless, it should be noted that the difference between the best and poorest sorts of this group for bread making is fully as great and sometimes greater than between the former and some varieties of other groups. The hard, red-grained varieties are by far the best both in food content and for our present system of roller milling. They include the Fifes, Velvet Blue Stem, Turkey, Mediterranean, and Fulcaster, of this country and Canada; the Ghirkas, Ulka, Crimean, and Buivola, of Russia; and the Theiss and Banat, of Hungary and Roumania. On the other hand, the white wheats and soft winter wheats give the best success in the manufacture of crackers. Several of the most popular breakfast foods are also made from white wheats. In a few instances macaroni is made from the hard spring wheats and the white wheats, but not extensively. No varieties of the bread-wheat group are well adapted for this purpose.

The special qualities that are found in varieties of this group may be summarized as follows:

(1) Excellence of gluten content for bread making.
(2) Excellence of certain varieties for cracker making.
(3) Yielding power of certain sorts.
(4) Rust resistance in some varieties.
(5) Hardy winter varieties.
(6) Resistance to drought (in some varieties).
(7) Early maturity (in some varieties).

CLUB OR SQUARE HEAD WHEATS (*T. compactum*).

By most writers this is not even ranked as a subspecies, but the different varieties certainly form an isolated group which is quite complete in itself and distinct from all other wheats, and which will therefore be considered here as a distinct species. The various varieties are commonly known under the names "club" or "square head". In this species the plant is very erect, with stiff, usually rather short, culm, attaining an average height of probably little more than 2 feet. The heads are extremely short as a rule, and often squarely formed, in some varieties much broader and flattened on the furrow side, usually thicker at the apex than at the base, commonly white but sometimes red, bearded or bald, the bearded varieties usually being native in hot countries. The spikelets are set extremely close together, often standing almost at right angles to the rachis (stem), three or four-grained, sometimes with four grains nearly throughout the entire head. The outer and inner chaff are much the same as in the bread wheats. The grains are usually short and rather small, white or red, often boat-shaped, and occasionally appear much like those of naked barley.

The peculiar structure of the head in this species allows the varieties to be comparatively large yielders, which is naturally their most

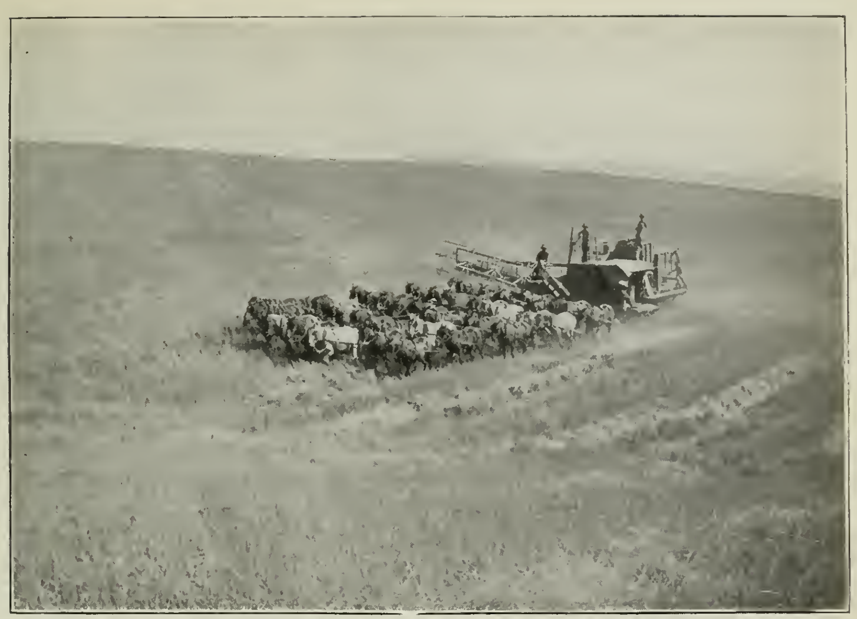


FIG. 1.—COMBINED HARVESTER-THRESHER AT WORK NEAR WALLA WALLA, WASH. (PHOTOGRAPHED BY A. B. LECKENBY.)

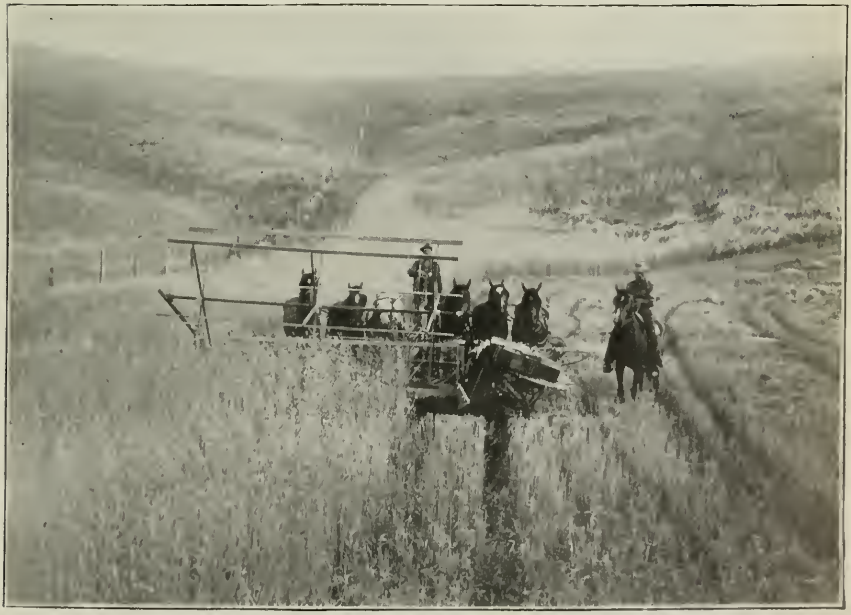


FIG. 2.—HARVESTING WITH THE WIDE-CUT BINDER NEAR COLFAX, WASH. (ORIGINAL.)

important quality. They are very deceptive in this regard, the shortness of the head leading one to suppose at first that it can not contain so many grains as are present in reality. The chaff is usually very tenacious, so that these wheats may be harvested long after ripening without loss from shattering. This is especially true of varieties grown in California and Washington. Having short, stiff straw, these wheats also usually stand up well, any damage from lodging being quite rare among them. Besides producing the class of flours desired in certain localities, club varieties are very good for cracker making and for the more starchy kinds of breakfast foods. They are grown either as spring or winter varieties except in Turkestan, where the winters are too cold for fall sowing. Being grown in dry, hot regions, they are usually rather drought resistant.

Club wheats are at present cultivated chiefly in the Pacific Coast and Rocky Mountain States of this country, in Chile, Turkestan, and Abyssinia, and to a slight extent in Switzerland, Russia, and a few other districts of Europe. The special qualities of the group are as follows:

(1) Great yielding power.
(2) Stiffness of straw.
(3) Freedom from shattering.
(4) Early maturity (in some varieties).
(5) Drought resistance (in some varieties).
(6) Excellence of certain varieties for cracker making and breakfast foods.

POULARD WHEATS (*T. turgidum*).

This group of wheats is usually classed as being quite distinct from the durum (*T. durum*) group, the two ranking as subspecies of *T. vulgare.* But as a matter of fact there are intergrading varieties which bring them as close together as are the club wheats and common bread wheats. They will both be considered here, like *T. compactum*, as distinct species.

The poulard wheats are usually rather tall, with broad, in most varieties velvety, hairy, or often glaucous leaves. The stems are thick and stiff, and sometimes pithy within. Heads long, often squarely shaped, with long beards, that are white, red, or bluish red in color, or sometimes black. Spikelets two to four-grained, and arranged rather compactly. Outer chaff strongly and sharply keeled. Grains large, proportionally short and rounded, sometimes almost semicircular in middle cross section, rather hard and glutinous, light yellowish red in color, sometimes nearly white, and becoming glassy in varieties allied to the durum group, or on growing in certain soils.

The name poulard is most commonly applied to these wheats. In Europe they are sometimes called English wheats, a very misleading name, as they are really little grown in England. On the other hand the few varieties that have been grown there are known as rivet wheats. A name often used in Germany is *bauchiger Weizen*, and a French name of corresponding meaning occasionally used is *blé petanielle.*

The wheats of this group are used sometimes in the manufacture of macaroni and other pastes. They are also occasionally used in bread making, but are more often employed for mixing with common bread wheats in grinding in order to give the quality of flour that is desired in the French markets.

To a small section of this species, having compound or branched heads, some have given the separate name of composite wheats (*T. compositum*). Some well-known varieties of this section are Seven-headed, Wonder Wheat, Hundred Fold, and Miracle. It should be noted, however, that the group of emmers (*T. dicoccum*) includes several varieties with compound heads similar to these. Many facts known in connection with the existence of these closely allied forms, together with that of the intergrading sorts between the poulards and durums, afford strong evidence of the occurrence of natural hybrids among the varieties of these three groups.

The poulard wheats are native usually in hot, dry regions, and are therefore often rather drought resistant, but not so much so probably as the durums. Many of the varieties are also very resistant to orange leaf rust. These wheats are grown chiefly in France, Egypt, Italy, Turkey, Greece, southern Russia, and other districts bordering the Mediterranean and Black seas. In this country they are only rarely grown; so far, in an experimental way. Special qualities of value to be found in this group are:

(1) Excellence of certain varieties for making macaroni.
(2) Resistance to orange leaf rust.
(3) Resistance to drought.
(4) Stiffness of straw.

DURUM WHEATS (*T. durum*).

As already stated, this group of wheats is rather similar to the poulard group. As a rule, however, the heads are not so thick and the grains are longer and much harder. The plants are rather tall, with stems either pithy within, or hollow with an inner wall of pith, or in a few varieties simply hollow as in the common bread wheats. The leaves are usually smooth, but have a hard cuticle, and are almost always resistant to orange leaf rust. The heads are rather slender, compactly formed, occasionally very short, and always bearded, with the longest beards known among wheats; spikelets two to four-grained. The outer chaff is prominently and sharply keeled, and the inner chaff somewhat compressed and narrowly arched in the back. The grains are usually very hard and glassy, sometimes rather transparent, yellowish white in color, occasionally inclining to reddish, and proportionally rather long. In the variety Arnautka the grains are almost or fully as large as those of Polish wheat, and are sometimes actually mistaken for the latter.

The varieties of this group are generally best known as the durums. In Europe they are often called, and correctly so, simply hard wheats. They are the hardest-grained wheats that are known. The Fifes, Velvet Blue Stem, Turkey, and others of that class usually called hard wheats in this country are not, strictly speaking, hard wheats at all when compared with these. On account of the resemblance of the heads of these wheats to those of barley they are sometimes called barley wheats or *Gerstenweizen*.

Durum wheats are particularly sensitive to changes of environment, and quickly deteriorate when grown in a soil or climate to which they are not well adapted. Such a change of conditions may be encountered, too, within the distance of a few miles. For example, it is well understood in south Russia that the excellent variety Arnautka gives the best results only when grown within a very limited area bordering the Sea of Azov. So also the best Kubanka is found east of the Volga on the border of the Kirghiz Steppes. In the Caucasus this variety has actually developed into a red winter wheat, though the original is a yellowish-white spring wheat.

The durum group furnishes the great bulk of the world's supply of macaroni wheat, though a considerable amount of these pastes is made from poulard and Polish varieties and a still smaller proportion from the common bread wheats. There is now not the least doubt that some if not all these durum sorts used for macaroni can be successfully grown in this country, thus adding immensely to the profits of our wheat industry. The success that has attended the trials of the variety Nicaragua in Texas has already conclusively proved the point. At the same time the idea that these wheats can not be successfully used for bread has never yet been shown to be more than mere assumption. Several mills in this country have successfully ground them, and in southern Russia, where milling has developed to a high degree of perfection, it is no longer an experiment. In that region durum wheat has become actually the most popular for bread making, though it is usually mixed with a small per cent of ordinary red wheat before grinding. In France there is an increasing demand for durum wheats for all purposes.

Durum wheats are adapted for soils rather rich in nitrogenous matter but somewhat alkaline, and give the best results in a very hot, dry climate. They are, therefore, quite drought resistant. Almost all varieties are adapted only for spring growing except in mild latitudes. The young plants both of this group and the poulard group are very light green in color at first and grow up rapidly. They are grown in Spain (where they predominate over all other groups) and other Mediterranean countries, in south and east Russia, Asia Minor, and to some extent in Mexico, Chile, and Argentina. In this country one variety, Nicaragua, is grown to a limited extent, chiefly in Texas.

The special qualities to be obtained in this group are briefly:

(1) Excellence of gluten content for making macaroni and other pastes.
(2) Resistance to drought.
(3) Resistance to orange leaf rust.

POLISH WHEATS (*T. polonicum*).

This group is considered by all writers to belong to a distinct species. Though there are several subspecies and varieties, apparently only one variety, White Polish, is very widely known. The plant is usually rather tall, with stems smooth and more or less pithy within. It does not stool extensively. The heads are extremely large and loosely formed, and before ripening are bluish-green in color. A special peculiarity of this species is the rather long, narrow, outer chaff, papery in structure, and standing out slightly from the head, instead of being rigid and closely applied to the spikelets, as in other wheats. The grains are of great size when normal, proportionately quite long, yellowish-white in color, and very hard. The name Polish wheat is universally applied to this species, though for what reason it is not clear. There is no evidence at all that it originated in Poland, and in fact it has been very little grown in that region. It is more probable that its native home is some portion of the Mediterranean region. A red winter wheat grown rather extensively in Poland and southwest Russia and also called Polish wheat, should not be confused with this group, as it is radically different, being one of the bread wheats. Other names have been given to this species but they are quite local in their use; such are Giant rye, Astrakhan wheat, Jerusalem rye, etc.

In almost all of the few cases where Polish wheat has been tried in this country it has proved a success from both the standpoint of yield and quality of the grain. But it seems never to have occurred to anyone to make use of the wheat for the production of American macaroni, though no doubt it would be excellent for that purpose, and a great demand for its increased production could thus be created. As it is, there is not sufficient incentive to the farmer for growing this wheat, since it is not well adapted for bread making if used alone.

Though requiring considerable moisture at seed time, Polish wheat is admirably adapted for cultivation in arid districts; in fact, it produces the best quality of grain when grown under arid conditions. It is also somewhat resistant to orange leaf rust, but not so valuable in this respect as the durum wheats. Varieties of this species are grown chiefly in Italy, Spain, and other portions of the Mediterranean region, and in southern Russia and Turkestan. They are also said to be cultivated to some extent in Brazil.

The special qualities of value belonging to Polish wheat are similar to those of the durum group, and are as follows:

(1) Quality of gluten content for making macaroni.
(2) Resistance to drought.
(3) Resistance to orange leaf rust.

SPELT (*T. spelta*).

This and the two following species are in several respects very different from any of the preceding groups. They are also not widely cultivated, although more commonly grown than Polish wheat, and are used only to a very limited extent for human food. Nevertheless, in the intercrossing of wheat groups for the improvement of our bread wheats some very valuable qualities may be obtained from varieties of these species.

The varieties of this group are called spelt in English, *Spelz* in German, and *épeautre* in French. In Germany the old name *Dinkel* is also sometimes applied. The varieties often called spelt in this country and Russia are not spelt, but emmer (*T. dicoccum*).

The spelt plant (Plate VII) grows to the average height of wheat, or perhaps a little higher, and possesses a hollow stem. The leaves are of ordinary size, usually smooth, but sometimes with scattering hairs; heads loose, narrow, and rather long, bearded or bald, especially characterized by a very brittle rachis, allowing them to be easily broken in pieces in thrashing. The spikelets are usually far apart in the head, arched on the inner side, and contain usually two grains; outer chaff oval, four-angled, boat-shaped, and only slightly keeled; grains light red in color, somewhat compressed at the sides, with a narrow furrow, the walls of the furrow flattened, and with sharp edges. The grain is always held tightly within the chaff, and can not be hulled in thrashing.

Spelt is used very little for human food, but is generally fed to stock. It is very important, however, for certain portions of our country, at least, to obtain for the bread wheats the particular quality of this group of holding the grain tenaciously. This can readily be done, as the Garton Brothers have amply demonstrated in England, by intercrossing varieties of the two groups. For certain varieties that would otherwise be of great value in the Pacific Coast and Rocky Mountain States such an improvement of preventing shattering at harvest is the most important that can be made. The few varieties possessing this quality that are now grown in these districts are sometimes not desirable in other respects. At the same time complaint is often made that certain introduced varieties which are most excellent from the standpoint of yielding capacity and hardiness are rendered worthless because of the great loss from shattering. It has also been observed by certain experimenters that the quality of constant fertility, or of producing "well-filled" heads, is greatly increased by the introduction of the spelt element. No doubt we very little realize the loss in yield that is simply the result of the inability of the variety to fill out its heads.

There are both winter and spring varieties of spelt, and some of the former are very hardy. Certain varieties are also rather drought resistant, but nearly all sorts are more or less susceptible to rust attacks. It is in just such cases as that of the use of spelt varieties in intercrossing with bread wheats that the greatest of judgment must be exercised because of the presence of undesirable as well as desirable qualities. While the experimenter is endeavoring to secure the qualities of nonshattering, drought resistance, etc., it is equally important to select from the progeny of such crosses in such a way as to eliminate the characteristics of rust liability and brittleness of the head. Here also is shown emphatically the advantage of the practice of composite crossing (to be discussed further on), as in such case the variation induced is so great that there are almost certain to be individuals present among the sporting offspring which possess the desired qualities without having preserved the undesirable ones.

Spelt is chiefly grown in Germany, Italy, Spain, France, and Switzerland, and perhaps to a small extent in Brazil. It is not grown in this country except mainly in an experimental way. Summarized, the desirable qualities found in the spelt group are:

(1) Power of holding the grain in the head.
(2) Constancy in fertility.
(3) Hardiness of certain winter varieties.

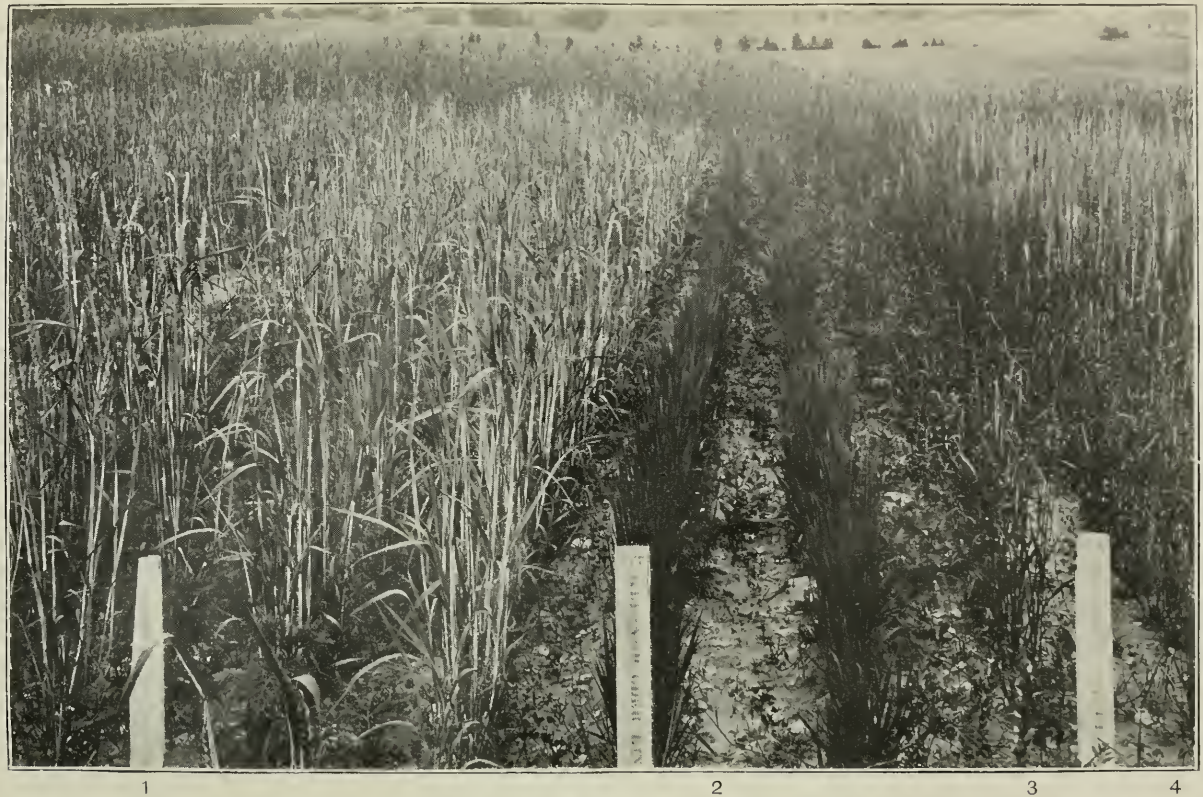
The undesirable ones are:

(1) Brittleness of the head.
(2) Rust liability.

EMMER (*T. dicoccum*).

This species has no English name, but is often incorrectly called spelt in this country. The German name is *Emmer* and the French *amidonnier*. As the German name is best known and easily pronounced, it should be at once adopted with us, and the name spelt applied where it properly belongs. In Russia, where the group is well represented, it goes by the name of *polba*, which name is invariably translated spelt. But either the Russians wrongly apply the name polba or this is an incorrect translation. As a matter of fact, very little if any true spelt is grown in Russia, though a rather large quantity of emmer is produced each year.

The plants of this species are pithy or hollow, with an inner wall of pith; leaves sometimes rather broad, and usually velvety hairy; heads almost always bearded, very compact, and much flattened on the two-rowed sides. The appearance in the field is therefore quite different from that of spelt. The spikelets (that is, the unhulled grains as they



SPELTS AND EINKORNS IN EXPERIMENTAL PLATS AT GARRETT PARK, MD.: 1, SPELTS; 2, EINKORN; 3, ENGRAIN DOUBLE; 4, SPELTS.
(ORIGINAL.)

come from the thresher), however, look considerably like those of spelt, but differ principally in the presence always of a short pointed pedicel. This pedicel, which is really a portion of the rachis (stem) of the head, if attached at all to the spelt spikelets, is always very blunt and much thicker. Besides, the emmer spikelets are flattened on the inner side, and not arched as in spelt, so that they do not stand out from the rachis as the spelt spikelets do, but lie close to it and to each other, forming a solidly compact head. The spikelets are usually two-grained, one grain being located a little higher than the other. The outer chaff is boat-shaped, keeled, and toothed at the apex. The grain is somewhat similar to that of spelt, but is usually harder, more compressed at the sides, and redder in color.

For the production of new varieties by hybridization emmer has qualities similar to those of spelt, but still more valuable. At the same time emmer, besides possessing harder grain, is more resistant to drought, and usually rather resistant to orange leaf rust. It is well adapted for cultivation in the northern States of the Plains and has already proved very valuable as a hardy forage plant in that region, besides giving a good yield of grain per acre. Almost all varieties are spring grown. Of other countries emmer is chiefly cultivated in Russia, Germany, Spain, Italy, and Servia, and to some extent in France. The emmer of this country is descended from seed originally obtained chiefly from Russia, where a considerable portion of the food of the peasants of the Volga region is a sort of gruel ("kasha") made from hulled and cracked emmer.

The desirable qualities furnished by this group of wheats are:

(1) Power of holding the grain in the head.
(2) Drought resistance.
(3) Resistance to orange leaf rust.

The undesirable qualities are:

(1) Brittleness of the head.
(2) Adaptability only for spring sowing.

EINKORN (*T. monococcum*).

This species of wheat is very distinct from any of the others, though the heads resemble those of emmer somewhat. It has no English name, but is called *Einkorn* in German and *engrain* in French. The German name is adopted here.

Einkorn (Plate VII) is a short, thin, and narrow-leaved plant, which presents a peculiar appearance in the field. It seldom reaches a height of more than 3 feet. The stem is hollow, thin, and very stiff. The leaves are usually quite narrow, sometimes hairy. Those of the young plant are sometimes bluish-green, and after flowering the plant becomes yellowish-green. Portions of the stem may also be brown. The heads are slender, narrow, very compact, bearded, and much flattened on the two-rowed sides, and always stand erect, even when ripe, but break in pieces easily. The spikelets are flat on the inner side, or form a concave surface with the projecting edges of the outer chaff. They are arranged very compactly in the head and are usually one-grained, except in the variety Engrain double (Plate VII), where they possess two grains. The outer chaff is deeply boat-shaped and rather sharply keeled, the keel terminating in a stiff tooth. The grains, which are tightly inclosed in the spikelet, are light red and extremely flattened, becoming thus bluntly two-edged and possessing an exceedingly narrow furrow.

This species is at present but little improved over the original wild form, and only a few varieties have been developed. Nevertheless some of the most valuable qualities may be expected from these varieties if they can be successfully employed in hybridization experiments. They are among the hardiest of all cereals and seem to be constant in fertility, and in the writer's experience are absolutely proof against orange leaf rust. Einkorn is entirely unknown in this country, except among a few experimenters, but is grown to a limited extent in Spain, France, Germany, Switzerland, and Italy. The two chief varieties are common Einkorn and Engrain double.

The valuable qualities to be obtained in this species may be summarized as follows:

(1) Power of holding the grain in the head.
(2) Resistance to orange leaf rust.
(3) Hardiness.
(4) Resistance to drought.
(5) Stiffness of straw.

An undesirable quality is:

(1) Brittleness of the head.

GEOGRAPHIC GROUPS OF WHEATS.

From the description of the different natural groups just given and the statements concerning their geographic distribution, it may be inferred that the localities as well as the natural groups might also be given from which particular qualities in wheat can be obtained. This can be done, but not with the completeness that could be desired, as it is not yet accurately known what kinds of wheat grow in all regions of the world. However, the matter may be stated approximately and briefly as follows: (1) White wheats containing much starch are grown in the Pacific Coast and Rocky Mountain States of this country, in Chile, in Turkestan, and to some extent in Australia and India. (2) Amber or reddish-grained wheats, also starchy, are to be found chiefly in the Eastern States of this country, in western and northern Europe, and to some extent in India, Japan, and Australia. (3) Large proportions of gluten content of the quality considered to be necessary for making the best bread are found in the red wheats of Canada and our Northern and Middle States of the Plains, in eastern and southern Russia, in Hungary and Roumania, and in southern Argentina. (4) Resistance to orange leaf rust is to be secured in the bread wheats of southern Russia (particularly in the Crimea and Stavropol government), in the poulards, emmers, and einkorn of the countries bordering the Mediterranean and Black seas, and in a few varieties in Australia. (5) Large gluten content of the quality necessary for making the best macaroni is furnished by the durums, poulards, and Polish wheat of Algeria, Italy, Spain, and especially of the northern shores of the Black and Azov seas in Russia, and to a limited extent in the State of Texas in this country. (6) Stiffness of straw, preventing the lodging of the grain, is found in the einkorn and some of the spelts, durums, and poulards of the Mediterranean countries, and in the dwarf bread wheats of Japan, and some of the club wheats of our Pacific Coast States, Turkestan, and Australia. (7) Great yielding power, at least in proportion to the length of the head, is obtained in the club wheats of the Pacific Coast States of this country and Chile, and Turkestan. (8) The quality of holding the grain, or nonshattering, is found in the club wheats of the Pacific Coast States, Chile, and Turkestan, and in all the spelts, emmers, and einkorn of east Russia, Germany, and the Mediterranean countries, and to a limited extent in the emmers of our northern States of the Plains. (9) Constant fertility, so far as known at present, is probably best obtained in the spelts of Germany and Southern Europe. (10) Early maturity is found to a limited extent in some of the bread wheat varieties of Australia and India, and in the dwarf wheats of Japan. (11) Resistance to drought and heat is best secured in the common red wheats and durums of south and east Russia, and the Kirghiz Steppes, the durums of the south Mediterrean shore, and both the bread wheats and durums of Turkestan. (12) Resistance to drought and cold is found to the greatest degree in the red winter wheats of East Russia.

IMPROVEMENTS ACCOMPLISHED.

Looking to the future, the possibilities for wheat improvement appear to be unlimited, and it is with these that we are of course more directly concerned at present. It will be of interest, however, to consider briefly some of the great changes for the better that have already been made in the wheat industry of this country during its short history. Some of these changes have been accomplished in line with similar ones in other countries, and have been coincident with improvements in the milling process or with the demands of consumers for greater variety in food, but in the main they have followed as a natural result of the development of the country. As wheat is not native in the United States, necessarily all seed was originally brought from other regions. At first, the territory being limited and the demands of the people comparatively simple, very few varieties were sufficient; but as the country rapidly developed and new territory was from time to time added and thrown open to settlement, new and varied conditions of soil and climate were encountered, and to meet the requirements of these new conditions other new and different varieties became necessary in order for the best success.

INTRODUCTION OF NEW VARIETIES.

A full history of the introduction of new varieties of wheat into this country, and from one section of the country to another, would be a matter of much interest but can not be attempted here. Only a few of the most important instances will be mentioned—those that mark real epochs in the development of our wheat industry, and have in certain localities entirely revolutionized wheat culture.

By far the most important among the earliest varieties introduced is the Mediterranean wheat, obtained first in 1819 from the islands of the Mediterranean Sea. At various times after that date this Department secured seed of the same variety and distributed it to all parts of the country. It soon met with favor everywhere. It is a hardy, bearded variety, productive, and producing a large red grain of good milling quality. But more than all this it was found to be quite resistant to rust and to damage by the Hessian fly, two enemies of the wheat crop which had already begun to be very much dreaded. This wheat has maintained its excellence through all decades since, and is to-day one of the most popular varieties in certain States, particularly Texas. It has also been used as a parent of several very valuable hybrids.

A most interesting example of improvements that are possible in the adoption of varieties best adapted to a particular region is found in the Fife wheats of Canada and the Northern States of the Plains. These varieties, which have become the basis of the great wheat and flour production of the Northwest, originated, according to the Canadian Agriculturist of 1891, in the following manner:

> Mr. David Fife, of the township of Otonabee, Canada West, now Ontario, procured through a friend in Glasgow, Scotland, a quantity of wheat which had been obtained from a cargo direct from Dantzic. As it came to hand just before spring seed time, and not knowing whether it was a fall or spring variety, Mr. Fife concluded to sow a part of it that spring and wait for the result. It proved to be a fall wheat as it never ripened, except three ears, which grew apparently from a single grain. These were preserved, and although sown the next year under very unfavorable circumstances, being quite late and in a shady place, it proved at harvest to be entirely free from rust when all wheat in the neighborhood was badly rusted. The produce of this was carefully preserved, and from it sprang the variety of wheat known over Canada and the Northern States by the different names of Fife, Scotch, and Glasgow.

If the above is an accurate statement of the introduction of Fife wheats, indications are rather strong that they are of Russian origin, judging from the description of the grain and source of the cargo, in connection with the present similarity of these wheats to Russian varieties. Their subsequent history in the Northwest and the impetus given to the wheat industry of that region through their cultivation are well known to agriculturists generally. Various strains have been developed till there are now a dozen or more so-called varieties in use. They are red, hard-grained wheats (as we use the term) similar to the Ghirkas of the Volga region, yield fairly well, and produce flour of excellent quality.

In Michigan there has been an energetic movement for a decade or longer to obtain hardy winter sorts, which has resulted in a great improvement not only for that State but for adjoining territory. The millers of the State have especially been active in this movement and the matter has been frequently a prominent topic of discussion at the meetings of the State Millers' Association. Budapest from Hungary and Dawson's Golden Chaff from Canada have been introduced and become favorite varieties. Another variety, Theiss, introduced from Hungary, has obtained a well-merited reputation as a hardy, red winter sort in the North Central States and as far west as Kansas. It has, however, not even yet received the attention that it should have.

Perhaps the most remarkable development in wheat culture in this country has been made in the Middle States of the Plains, in what we may now call the Hard Winter Wheat district, all brought about through the introduction of the hardy, red-grained wheats. Twenty-five years ago very little hard wheat was grown in this region, the seed being brought by the early settlers from States farther east, where soft wheats were chiefly cultivated. Also, spring varieties formed the basis of a large proportion of the wheat production. But the spring wheats were severely rusted, injured by drought because of late maturity, and in some seasons almost wholly destroyed by chinch bugs, while the soft winter sorts, such as White Michigan and Poole, also rusted badly and were not able always to stand the winters. For some time these defects were overcome in great measure by the use of the variety Odessa, popularly called "Grass" wheat in some localities, which is probably equivalent to the variety Ulka of southern Russia. It is hardy, red-grained, rather rust resistant, and has the additional advantage of being adapted for either autumn or spring sowing. A little later, the well-known variety Fultz also became quite popular in the West, as it is still in the greater portion of the United States.

But the variety which more than all others finally completely changed the status of wheat culture in this district, is that which is commonly but unfortunately known as Turkey. It is a bearded, hard red wheat of the highest class, coming originally from the Crimea and other portions of Taurida in southern Russia, and not from Turkey as the name would imply. Within a very small area in Kansas, Turkey wheat has been grown about twenty-five years, but its merits have become generally known only during the last twelve or fifteen years. It is now a favorite variety in the middle Great Plains. By the use of this variety autumn sowing is now made practicable in most seasons to the northern limit of the district, and the winter-wheat flour from this region has obtained a reputation for quality of the very best, and distinctively its own, in the foreign markets. At the same time there is no longer so much damage resulting from the attacks of rust and chinch bugs. As it is also one of the most drought-resistant sorts, it has made it possible to extend the winter-wheat area farther westward as well as northward.

In a large part of the Pacific coast region, including the Palouse country, the improvements which have resulted in such large yields and great profit in certain localities were made chiefly through the introduction of club wheats, which are very productive, hold the grain in the head, and are in other regards well adapted to the conditions of the region. One or more of these wheats came originally from Chile, and others probably from Australia and France, but the origin of many of them is not accurately known. Two other varieties, not club wheats, namely, Australian and the Palouse Blue Stem, are also two of the most valuable wheats of this district and probably belong to the Purple Straw group of Australia.

In southern California and the Irrigated Wheat district the variety Sonora has had the greatest influence in the development of wheat culture. It is a white-grained sort with reddish, velvet chaff, but the grains are a little harder than those of the club type and better adapted for export. It came originally from the State of Sonora in Mexico.

Among later introductions is the variety called Nicaragua, a durum wheat, already discussed in another part of this bulletin, which is likely to take a considerable part in the future wheat production of this country, both because of its adaptation—as is true of all durum varieties—to the hot, dry summers of the southwestern Great Plains, and because of its suitability for the manufacture of macaroni. No facts concerning the origin of this variety are at present known to the writer, though one would infer from the name that it came from Nicaragua, and it is true that varieties of the same group are known in that country. It has been known in Texas for many years, and its use has made it possible to grow wheat in portions of that State not before successful in wheat growing. A variety similar to this one, called Wild Goose, is grown to a very limited extent in North Dakota, and probably came originally from southern Russia. It is also likely to be of value for the production of macaroni, though it seems to be somewhat inferior to Nicaragua.

WORK OF THE DEPARTMENT.

In connection with the discussion of the introduction of varieties, it is hoped that it will be of interest to give an account of the experiments made by this Department with wheats from all parts of the world. Though the aim in beginning these experiments, as already stated, was primarily to test rust resistance, the work naturally soon developed into a study of the characteristics in general of the varieties of different natural groups of wheats, and of groups considered geographically, and some most interesting facts were thus obtained which will be of great value in the work of wheat improvement. In fact, many statements made in the foregoing discussion of the "Sources for desirable qualities" are based upon the results of these experiments. Varieties were obtained from every wheat country of the world, aggregating about 1,000 rather distinct sorts in all. The manner of securing these wheats, and the time and labor thus involved, together with the difficulties of nomenclature arising from the confusion of varietal names which prevails generally, have all been discussed in a former bulletin on Cereal Rusts of the United States, and need not be referred to here. The varieties were grown one year in Maryland and most of them one year in Kansas, while about 300 of them were grown two years in Kansas, or three years in all; that is, during 1895, 1896, and 1897. During the same time a number of the varieties, especially from Russia, Siberia, Japan, and Argentina, were tested by other parties in cooperation with the Department, in other localities, viz, in Michigan, Wisconsin, Indiana, Tennessee, and Colorado; and in the case of a few of these, the experiments have since then been repeated in Colorado, Kansas, and Nebraska.

In conducting these experiments all the principal characteristics of the wheat plant, as shown in its different stages from that of the young plant to harvest time, were studied, though complete notes can not be given for every variety. These features include in a general way (1) the character of the young plant; (2) hardiness, including resistance to rust, drought, and cold; (3) character of the head; (4) character of the grain; and (5) time of maturity. Field experiments alone do not show those qualities of the grain which indicate the value of the variety for different uses, and which are after all more important than any others; though it must be remembered that certain characteristics of the growing plant often indicate quite correctly what these qualities will be. Therefore chemical analyses have been made of a large number of representative varieties, and for many of them the absolute weight and specific gravity of the grain have also been determined.

As would be expected, a large number of the varieties either proved to be entirely unsuited to the conditions in this country or were found to be in other respects undesirable sorts. It was the purpose from the start to discard gradually all varieties that seemed to be unable to adapt themselves to their new environment. During the first year of the experiments at Garrett Park, Md., many of them were planted so late, on account of their late arrival in this country, that much allowance must be made for their behavior in comparison with others which were sown in good season. In other respects the trial for that year was very satisfactory, and afforded an excellent opportunity of comparing the behavior and qualities of a large number of varieties under average conditions.

But the larger portion of our area most important in wheat production lies much farther westward than Maryland and possesses a very different soil and a climate characterized by great extremes of drought, cold, and heat. At the same time it is manifestly desirable to search particularly for varieties adapted to such extreme conditions for two reasons: (1) It is found that as a general rule sorts which are able to withstand the most rigorous extremes of climate are also of the class which makes the best quality of bread and macaroni, the two principal purposes for which wheat is used.[1] (2) It is comparatively easy to obtain varieties suitable for mild conditions, as those which are resistant to climatic extremes are more easily grown in a milder climate than the reverse. It was therefore decided to test the varieties during the following years in the Great Plains. Accordingly, in 1896 the field experiments were carried on at Salina, Kans., and in 1897 at Manhattan, Kans. At Salina Mr. B. B. Stimmel kindly donated the use of about two acres of land for the experiments. At Manhattan, by courtesy of the board of regents of the Kansas Agricultural College, the farm department of the experiment station was authorized to cooperate with this Department in the experiments conducted at that place, by furnishing land and other facilities for the work.

During the years of the experiments in Kansas the seasons were unusually severe even for that region. As a result it was found desirable to discard a large number of sorts from year to year. Only about 300 varieties were grown at Manhattan in 1897 out of the 1,000 originally obtained, and of these, less than 200 were selected as being worthy of continued trial. Over 100 of the varieties finally remaining have been made the basis of a large part of the series of field experiments now in progress at Halstead, Kans., while a number of the same varieties are still being tested at the Nebraska Experiment Station Farm, Lincoln, Nebr., and at the Arkansas Valley Experiment Station, Rockyford, Colo. Through this process of rigid elimination, which is a good example of the practical application of the law of the "survival of the fittest" in agriculture, about 100 varieties have been determined upon as being fairly representative sorts of the world as regards hardiness and quality of gluten content. There are many varieties, however, which can not be classed with these hardy, glutinous sorts, but which, nevertheless, because of their early maturity or particular adaptation in other respects to certain localities where hardiness is not necessary, and where these sorts would fail because of the lack of other qualities, must be considered as equally important. Palouse

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[1] For the present, for the want of space, a full discussion of this proposition in detail can not be given, although the experimental proof concerning the qualities of varieties originating in regions of different climatic conditions will be brought out in connection with the table presented in the following pages. The whole matter is one of much interest and may be discussed in detail in another publication, The Relations of Soil and Climate to Wheat Production.

Blue Stem, Australian, Little Club, Early May, Allora Spring, Yemide, King's Jubilee, Early Genesee Giant, etc., are examples of this class. Adding still to these a number of other sorts, belonging to the Spelt, Emmer, and Einkorn groups, which are also hardy, but are especially valuable for certain qualities they may furnish in the work of hybridization, and then still a few others, mentioned favorably by other experimenters, we have in all 245 wheats which may be regarded as leading varieties of the world.

For comparative study the principal qualities of these 245 varieties are briefly stated in the following table, which is based mainly upon investigations of this Department, but to some extent also upon the work of others. As regards the field work, it represents a summary of the combined results of the three years' experiments, so that each column shows as nearly as can be given an average of that quality for each variety for the three years.[1]

The table is made up of twenty-five columns, and the information given in each is in most cases clearly explained by the heading, but in a few cases a little further explanation is perhaps needed. In column 2, the following abbreviations are used: C. for common or bread wheat; Cl. for club; D. for durum; P. for poulard; Pol. for Polish; S. for spelt; Em. for emmer; and Ein. for einkorn. In column 8, "stand" refers to the degree of completeness with which the plants fill the drill row, and of course often measures, though not always, the stooling quality of the variety. In column 9, under "spring condition," each number expresses in a scale of 1 to 100 the general condition of the variety in all respects about May 1. The figures in column 10 are percentages showing the comparative amount of leaf rust on the plant at the date when this rust was most abundant. This column is in the main a reproduction of the column of averages in Bul. No. 16 of this Division, Cereal Rusts of the United States, pages 26–32, table 3. Of course the smallest number represents the greatest degree of rust resistance. In column 11, the abbreviations C. and D. indicate that the variety corresponding is resistant to cold or drought and the figure shows on a scale of 1–5 how great is the degree of hardiness, 5 meaning extremely hardy. In column 19, the word "vitreous" refers to grain which is not only very hard but is somewhat transparent and presents a glassy surface in fracture. Wheats so characterized are usually durums. In column 25 is shown the particular wheat district of the United States to which the variety is best adapted. The districts are indicated by roman numerals having the following significations: I, Soft Wheat district; II, Semi-Hard Winter Wheat district; III, Southern Wheat district; IV, Hard Spring Wheat district; V, Hard Winter Wheat district; VI, Durum Wheat district· VII, Irrigated Wheat district, and VIII, White Wheat district.

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[1] There is one exception. In column 9, "spring condition," the data given refer only to the experiments of 1894–95 in Maryland.

Table presenting a comparative résumé of the principal qualities of 245 representative wheats of the world.

Name of variety.	Group.	Spring or winter.	Where obtained.	Character of growing plant.					Hardiness.		Time of heading.	Character of the head.		Time of ripening.
				Autumn condition.				Spring condition.	Resistance to leaf rust.	Resistance to cold or dr'ght.		Bald or bearded.	Other characteristics.	
				Color.	Manner of growth.	Amt. of growth.	Stand.							
Algerian	D.	S.	Algeria	Lt. gr.	Erect, nar. leaf	Lrg. tall		91	21	D. 5.	May 31	Bearded	Short heads, long beards.	June 22
Allora Spring	C.	W.	Australia	do	Erect	Large	Good	13	45		May 30	(*b*)		
Alsace	C.	S.	Russia	Gray gr.	Erect, velvety.	do	do	96	24	D	June 10	Bald		
American	C.	W.	United States	Dark gr.	Sprdg., fine lf.	Very lrg	do	91	67		May 26	Bearded		June 24
American Bronze	C.	W.	do											
Ames	C.	W.	Russia	Green	Erect, nar. leaf	Large	Good	90	(*a*)	D. 4.	May 31	Bearded		
Amethyst	C.	W.	Colorado		Pntd. nar. lvs., erect.			92	30		June 4	Bald		
Arnautka	D.	S.	Russia	Lt. gr.	Erect	Large	Poor	91	(*a*)	D. 5.	do	Bearded		June 23
Arnold's Hybrid	C.	W.	United States	Yel. gr.	Nar. lvs. sprdg	do	Fair	89	53		May 21	Bald		Do.
Assiniboine Fife	C.	S.	Canada	Lt. gr.				97		D. 3.	June 1	do		
A six rangs			France	Green	Coarse, sprdg.	Medium	Poor		26			Bearded		
Atalanti	D.	S.	Greece	Purplish	Nar. leaves	Large		77	(*a*)	D	May 31	do		Do.
Australian	C.	W.	California	Lt. gr.	Drooping			92			June 11?	Bald		
Australian Indian	C.	W.	Australia	Green		Large	Good	92	25		May 10			June 20
Australian Purple Straw	C.	W.	do					91			May 31	Bald		
Baggi	C.	W.	India	Lt. gr.	Slender	Tall, lrg		67			May 14	do		June 22
Banat	C.	W.	Hungary	Green	Spreading	Medium	Fair	71	24	C.2, D.3	May 27	Bearded		June 23
Barletta	C.	W.	Argentina	Lt. gr.	Erect	Large	Good							
Basalt	C.	W.	Colorado	do	Spreading			72			June 4	Bald	Velvet chaff.	
Bearded Winter	C.	W.	Russia	Dark gr.	do	Medium	V'y poor		50	C.4, D.4	June 6			
Bellevue Talavera	C.	W.	France	Green	do	do	do	68	38		June 4	Bald		
Berthoud	C.	W.	Australia	do	do	do	Fair	72	38		June 1	do	Red chaff.	
Belokoloska	C.	S.	Russia	do	Erect	Large	Good		28		June 5	do		
Beloturka	D.	S.	do	Lt. gr.	do	Medium	Fair	36		D. 5.	June 1	Bearded		
Bianchetta		W.	Italy	Green	Spreading	Small	V'y poor		53		June 9	Bald		
Big English	C.	W.	United States	do	Sprdg., fine lf.	Medium	Fair	90	50		May 26	do		
Big Frame	C.	W.	do			Tall, lrg		95	58		May 25	do		
Black Velvet	Em.		Germany						(*a*)			Bearded		
Blount's Fife	C.	W.	Colorado		Spreading			48			June 11	Bald		
Blount's No. 10	C.	W.	do		Drooping			91			May 27	do	White chaff.	
Blue Stem	C.		United States					91	53		June 7	do		
Bolton's Blue Stem	C.	S.	do											
Buca Nera	C.		Germany						(*a*)			Bearded		
Buckeye	C.	W.	United States	Green	Spreading	Very lrg	Good	92	54		May 27	Bald		Do.

a Almost free.

b Semibearded.

Table presenting a comparative résumé of the principal qualities of 245 representative wheats of the world—Continued.

Name of variety.	Character of the grain.								District of United States to which best adapted.	Remarks.
	Size.	Color.	Hardness.	Physical qualities.		Per cent of nitrogenous matter.				
				Abs. wt. 100 gr.	Sp. gr.	Albuminoids.	Gluten.			
							Moist.	Dry.		
Algerian	Large	Yel'sh wh	Vitreous						V, VI, VII	
Allora Spring	Sm. or med	do	Soft			12.06	22.78	8.70	III	Plant short.
Alsace	do	Reddish	Hard			*14.88 †11.56	*36.80 †26.50	*13.58 †9.30	IV	*Sample from Ekaterinoslav. †Sample from Poltava.
American			S. or semih						I, II	
American Bronze			Soft	3.908		11.03	24.78	9.46	I, II	
Ames		Red	Hard						V	Lodged slightly.
Amethyst	Med. or sm	White	Soft			*13.98	*32.61	*12.03	III	*Australian sample.
Arnautka	Large	Yel'sh wh	Vitreous			*16.13 †14.00	*33.11 †37.70	*13.04 †13.55	V, VI, VII	*Sample from Kharkov. †Sample from Poltava. Slightly lodged.
Arnold's Hybrid	Medium		Semihard						I, II	Lodged slightly.
Assiniboine Fife			Hard						IV	
A six rangs						10.69	18.38	7.42	I, II	
Atalanti	Large		Vitreous			14.38	31.72	11.65	VI, VII	Do.
Australian			Soft						VIII	
Australian Indian			do						III	
Australian Purple Straw			do						III, VIII	
Baggi	Medium	Light yel	do						III	Lodged badly.
Banat	Med. or sm	Red	Hard			 †14.88	*40.48 †32.58	 †11.60	II	*Sample from Hungary. †Sample from Russia. Lodged slightly; plant glaucous.
Barletta	Medium	Amber	Soft						I, II	
Basalt			do						I, III	
Bearded Winter	Small	Red	Hard			*15.38	*39.84	*13.87	V	Yield at Manhattan, Kans., 25 bu. per acre; in Colorado, 29½ bu. *Sample from Kharkov.
Bellevue Talavera			Soft			13.81	29.95	11.05	I, II	
Berthoud	Large	White	do							
Belokoloska	Medium	Reddish or amber,	Hard			*13.28 †14.19 ‡11.56	*32.95 †37.66 ‡24.20	*11.56 †13.35 ‡8.58	IV	*Sample from Kharkov. †Sample from Kursk. ‡Sample from Poltava.
Beloturka	Large	Yel'sh wh	Vitreous			*14.19	*34.61	*12.24	VI, VII	*Sample from Siberia. Lodged slightly.
Bianchetta										
Big English	Medium	Amber	Semihard						I, II	
Big Frame	do	do	do						I, II	Lodged badly.
Black Velvet									IV, V	
Blount's Fife	Medium		Soft			13.31	25.42	9.97	III, VII	
Blount's No. 10	do	White	do	4.690		9.45			III, VII	
Blue Stem	do	Red	Semihard						I, II	
Bolton's Blue Stem	Sm. or med	do	Hard	2.749	1.370				IV	
Buca Nera						14.19	26.67	10.63		
Buckeye	Medium	Amber	Soft						I, II	Lodged slightly.

Table presenting a comparative résumé of the principal qualities of 245 representative wheats of the world—Continued.

Name of variety.	Group.	Spring or winter.	Where obtained.	Character of growing plant.					Hardiness.		Time of heading.	Character of the head.		Time of ripening.
				Autumn condition.				Spring condition.	Resistance to leaf rust.	Resistance to cold or dr'ght.		Bald or bearded.	Other characteristics.	
				Color.	Manner of growth.	Amt. of growth.	Stand.							
Budapest	C.	W.	Hungary	Green	Spreading	Large	Fair	28	63	C.2, D.3	May 26			
Candeal Redondo	D.	W. or S.	Argentina							D				
Canning Downs	C.	W.	Australia								May 25	Bearded		
Cape	C.	W.		Lt. gr.	Drooping			81	58		May 31	Bald		
Cartagena			Spain						15					
Chernokoloska	D.	S.	Russia	Very lt. gr.	Erect	Very lrg	Good		15	D	June 7	Bearded	Short heads, black chaff.	
Chernouska		S.	do	Lt. gr.	Erect	Very vig	do	99	4	D	June 9	do		
Chiddam de Mars rouge	C.		France											
Chili			Chile								May 31	Bald		
Chili Club	Cl.		do											
China Red	C.	W.		Green	Sprdg., fine lf.	Lrg.; sht	Fair	91	38	C. 3	May 23	Bearded		June 21
China Tea	C.	W.				Large	V'y good	90	38	C. 3	June 4	do		
China White	C.			Green		V'y lrg; sh	Good	91	50	C. 3	May 25	do		Do.
Chinese	C.	S.	China	Lt. gr.	Erect	Large	Poor							
Chubut	C.	W.	Argentina	do	Erect, coarse					D		Bearded		
Clawson	C.	W.	United States	Green		Large	Good	94	51		June 4	Bald		June 23
Cretan								86	(*a*)		do	Bearded		
Crimean	C.	W.	Russia	Green	Spreading	Medium	Fair		48	C.4, D.4		do		
Currell's Prolific	C.	W.	United States	do	do	do	do	91	45		June 1	Bald		
Dallas	C.	W.	do	do				89	41		May 29			
D'Arblay's Hungarian	C.	W.	Hungary	do				65			June 6	B'd'd (?)		
Daruma	C.	W.	Japan	do	Spreading	Small	Fair	75			May 27	Bearded		
Dattel	C.	W.	Germany	do	Sprdg., fine lf.	do	do	92	39		June 7	Bald		
Dawson's Golden Chaff	C.	W.	Canada	do	Erect	Medium	do		75					
Defiance	C.	W.	United States					66			June 4	Bald		
Deitz	C.	W.	do	Green		Medium	Fair	93	50		May 31	Bearded		
De la Basse	P. (?)		Egypt					40		D	do	do		
Diehl Mediterranean	C.	W.	United States					89			do	do		
Diamond Grit	C.	W.	New York	Green		Large	Fair							
Dividenden	C.		Germany						43			Bald		
Duro di Apulia			Italy	Lt. gr.	Erect, sprdg		Poor		(*a*)		June 9	Bearded		
Early Arcadian	C.	W.	New York	Green		Medium	do							
Early Baart	C.	W.	Australia					76			May 27	Bearded		
Early Genesee Giant	C.	W.	New York											

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a Almost free.

Table presenting a comparative résumé of the principal qualities of 245 representative wheats of the world—Continued.

Name of variety.	Character of the grain.								District of United States to which best adapted.	Remarks.
	Size.	Color.	Hardness.	Physical qualities.		Per cent of nitrogenous matter.				
				Abs. wt. 100 gr.	Sp. gr.	Albuminoids.	Gluten.			
							Moist.	Dry.		
Budapest	Med. or sm	Red	H. or semih	2.836	1.331				I, II	
Candeal Redondo	Large	Yel'sh wh	Vitreous				35.76		VI, VII	
Canning Downs	Medium	White	Soft						III, VIII	Very short plant.
Cape	do									
Cartagena									VII, VIII	
Chernokoloska	Large		Vitreous			*15.75	*41.00	*14.68	VI, VII	*Sample from Kursk.
Chernuska	do		do			*15.94	*38.22	*14.10	VI, VII	Do.
Chiddam de Mars Rouge							10.50			
Chili			Soft							
Chili Club	Medium	Lt. yellow	do	*3.765 †4.536		*9.63 †10.50	*20.46 †22.06	*7.37 †8.19	VII, VIII	*Sample from Washington. †Sample from Oregon.
China Red	Small	Red	Hard						II, V	Lodged considerably.
China Tea	do	do	do	5.000		13.13	19.44	10.25	II, V	
China White	Medium								II	Do.
Chinese	do	Reddish	Semihard			9.44	21.20	7.80		
Chubut	do	Red	do	3.755	1.398	10.88	23.95	8.68	II	
Clawson	do	Amber	Soft	*4.242 †4.000		*10.68 †11.73	*23.35 †24.01	*8.91 †9.21	I, II	*Sample from Wisconsin. †Sample from Michigan.
Cretan	Large		Vitreous			*15.56	*42.57	*14.26	VI, VII	†Australian sample.
Crimean	Small	Red	Hard			15.25	38.97	12.91	V	Yield in Kansas 34½ bushels per acre.
Currell's Prolific	Medium	Reddish	S. or semih	3.098	1.316				I, II	
Dallas	do	Red	Semihard							
D'Arblay's Hungarian	do	do	Hard			14.94	31.04	10.13		
Daruma	do	Yel'sh br	Soft			10.13	21.50	8.15	III	
Dattel	do					*10.69 †13.31	*17.80 †27.63	*7.33 †10.49		*Sample from France. †Sample from Germany.
Dawson's Golden Chaff	do	Lt. yellow	Soft	3.203	1.302				I, II	
Defiance	do		do	*3.532		*13.13 †11.19	*30.90 *23.59	*11.95 †8.76	VII, VIII	*Sample from Maine. †Sample from Australia.
Deitz	do	Reddish	S. or semih							
De la Basse	Large			3.880	1.279					Plant extremely short.
Diehl Mediterranean			Semihard	*4.155 †4.186		*12.08 †14.18	*20.05 †27.51	*8.10 †10.85	I, II	*Sample from Michigan. †Sample from Indiana.
Diamond Grit	Medium	Reddish	do						I, II	
Dividenden						13.13	26.04	9.30	I, II	
Duro di Apulia										
Early Arcadian	Medium	Yel'sh br	Soft						I, II, VIII	
Early Baart			do						III	
Early Genesee Giant	Medium	Yel'sh br	do						I, II	

Table presenting a comparative résumé of the principal qualities of 245 representative wheats of the world—Continued.

Name of variety.	Group.	Spring or winter.	Where obtained.	Character of growing plant.					Hardiness.		Time of heading.	Character of the head.		Time of ripening.
				Autumn condition.				Spring condition.	Resistance to leaf rust.	Resistance to cold or dr'ght.		Bald or bearded.	Other characteristics.	
				Color.	Manner of growth.	Amount of growth	Stand.							
Early Japanese	C.	W.	Australia					70			June 1			
Early May	C.	W.	United States	Green	Spreading	Small	Poor	89	50		May 27	Bald		June 23
Early Red Clawson	C.	W.	do	do		Very lrg	Good	86			June 4	do		Do.
Early Rice	C.	W.	do	Dark gr.	Spreading	Medium	V'y poor	91	64		May 23			June 20
Einkorn	Ein.		Germany						Rust pr'f	C.5, D.5		Bearded	Nar. comp. hds	
El Safra	D.		Algeria	V'y lt. gr	Erect, coarse	Large	Fair			D		do		
Engrain double	Ein.		France						(*a*)	C.4, D			Nar. comp. hds	
Entre Rios	C.	W.	Argentina	Lt. gr	Erect	Medium	Good					Bearded		
Everett's High Grade	C.	W.	United States					90						
Farquhar	C.	W.	do	Green	Spreading	Very sm	V'y poor	91	68	Cold 3	May 28	Bald	Velvet chaff	
Farrer's Durum	D.		Australia	Lt. gr	Er., v'y nar. lvs	Large	Good	69	(*a*)		May 31	Bearded		
Felspar	C.	W.	Colorado					89				Bald		
Fern	C.		Germany					95	28		June 11	do		
Flourelle	C.		France	Green		Medium	Poor	90	73		June 1	do		
Fluor Spar	C.	W.	Colorado					66	47		June 4	do		
Frampton	C.	W.	do								June 6	do		
Frances	C.	W.	Argentina	Lt. gr	Erect	Medium	V'y poor	91	63		June 5	do		
Frankenstein	C.		Germany					90	50		June 4	do		
Fulcaster	C.	W.	United States	Green	Spreading	Medium	Poor	91	61	C.3, D.3	May 31	Bearded		June 24
Fultz	C.	W.	do	do	do	Very vig	Fair	95	69	C.3, D	May 27	Bald	White chaff	June 22
Galland's Hybrid	P.		France	Pea gr	Broad leaves			92	(*a*)		June 4	Bearded	Large	
German Amber	C.	W.		Green	Erect	Large	Good	92	60		May 27	Bald	White chaff	June 23
German Emperor	C.	W.		Dark gr.	Spreading	do	do	91	54		May 31	do		June 24
Gharnovka		S.	Russia	Lt. gr	Erect	do	Fair	98	11	D	June 11	Bearded		
Glyndon 673	C.	S.	North Dakota	do				95	60		June 1	Bald		
Glyndon 811	C.	S.	do									do		
Gold Coin	C.	W.	New York											
Golden Cross	C.	W.	do	Green	Spreading	Medium	Poor	92			May 29	Bearded		
Goldene Aue	C.		Germany					70	40			Bald		
Graf Walderdorff's Regenerated.	C.	W.	do						50			do		
Gypsum	C.	W.	Colorado	Green	Erect	Large	Good	57	33		June 1	do	White chaff	
Haffkani	C.	W.	Turkey					25	5		June 5	Bald(?)		
Hallett's Pedigree	C.	W.	England		Erect, nar. lvs.			95			June 4	Bald		
Hayne's Blue Stem	C.	S.	United States											

a Almost free.

Table presenting a comparative résumé of the principal qualities of 245 representative wheats of the world—Continued.

Name of variety.	Character of the grain.								District of United States to which best adapted.	Remarks.
	Size.	Color.	Hardness.	Physical qualities.		Per cent of nitrogenous matter.				
				Abs. wt. 100 gr.	Sp. gr.	Albuminoids.	Gluten.			
							Moist.	Dry.		
Early Japanese	Medium	Light br.	Soft						III	
Early May	do	Light red.	do						III	Lodged slightly.
Early Red Clawson	do	Red	do	*3.844 *4.285		*12.08 *12.25	*24.06 *26.36	*9.47 *10.15	I, II	*Sample from Indiana.
Early Rice	do		do						III	Lodged slightly.
Einkorn	Med. long	Reddish	Hard						IV, V	Plant short.
El Safra	Large	Amber yel	Vitreous						V, VI, VII	
Engrain double	Medium	Red	Hard						IV, V	
Entre Rios	do	Reddish	Soft	2.490	1.349	15.56	35.35	12.63	I, II	
Everett's High Grade	do								I, II	
Farquhar	do	Reddish	S. or semih						I, II	
Farrer's Durum	do	Yel'sh wh	Hard or vit			*14.77	*41.44	*14.49	V, VI	*Australian sample. Lodged slightly.
Felspar	do		Soft						III, VII	
Fern	do					11.56	21.67	8.10		
Flourelle										
Fluor Spar	Small		Soft			10.69	14.92	6.21	III, VII	
Frampton			do						III, VII	
Frances	Medium	Amber	do						I, II	
Frankenstein	do								I, II	
Fulcaster	Med. or sm	Red	Semihard.	*4.080 †4.987 ‡3.913		*12.78 †11.73 ‡14.53	*34.13 †26.34 ‡23.98	*12.74 †10.18 ‡9.59	II, III, V	*Sample from Pennsylvania. †Sample from North Carolina. ‡Sample from Kentucky.
Fultz	do	Reddish	do	*3.325 †2.828 ‡3.844	1.361	*11.55 †11.90 ‡12.25	*25.03 †27.18 ‡29.13	*9.88 †10.61 ‡11.33	I, II, III, V	*Sample from Michigan. †Sample from Indiana. ‡Sample from Missouri.
Galland's Hybrid	Large		Hard						VI	Plant velvety, hairy.
German Amber	Medium	Light am.							I, II	Lodged slightly.
German Emperor	do		Soft			*13.13	*30.14	*10.39	I, II	*Sample from Germany.
Gharnovka	Large		Vitreous			15.38	27.30	13.90	V, VI, VII	
Glyndon 673	Small	Red	Hard						IV	
Glyndon 811	do	do	do	2.747	1.377				IV	
Gold Coin	Medium	Light yel	Soft						I, II, VIII	
Golden Cross	do		do	3.718		10.50	22.20	8.65	I, II	
Goldene Aue			do			14.38	28.63	10.70	I, II	
Graf Walderdorff's Reg'n't'd			do			14.19	30.35	10.75	I, II	
Gypsum	Medium	White	do			10.35	19.41	7.54	III, VII	
Haffkani			Semihard			12.75	25.75	9.60	III	Plant very short.
Hallett's Pedigree			Soft	*3.880		*12.08	*22.90	*9.31		*Sample from Colorado.
Hayne's Blue Stem	Med. or sm	Red	Hard	2.644	1.346				IV	

Table presenting a comparative résumé of the principal qualities of 245 representative wheats of the world—Continued.

Name of variety.	Group.	Spring or winter.	Where obtained.	Character of growing plant.					Hardiness.		Time of heading.	Character of the head.		Time of ripening.
				Autumn condition.				Spring condition.	Resistance to leaf rust.	Resistance to cold or dr'ght.		Bald or bearded.	Other characteristics.	
				Color.	Manner of growth.	Amount of growth	Stand.							
Herisson barbu	Cl.	W or S	France					63			June 11	Bearded	Short	
Herisson sans barbes	Cl.		do									Bald	Red chaff, v'y short heads.	
Hickling									32			do		
Hopetown			England					92			June 1	do	Red chaff	
Hornblende	C.	W.	Colorado		Spreading			82	35		May 31	do		
Hudson's Early Purple Straw.	C.	W.	Australia						48		do	do	Purple straw	
Igel mit Grannen	Cl.		Germany					30	25		June 9	Bearded	Short	
Igel ohne Grannen	Cl.		do									Bald	do	
Imperial	C.	S.	Russia	Green	Erect	Very sm	V'y poor		55	D	June 16	do		
Improved Fife	C.	W.	Australia		Coarse			83	30		June 4	do		
Japanese, No. 1	C.	W.	Japan	Y'l'sh gr.	Sprdg narrow leaves.	Very lrg	Good							
Japanese, No. 2	C.	W.	do				do							V. early
Japanese, No. 4	C.	W.	do	Yel. gr.	Erect, coarse	Very lrg	V'y good							
Jejar de Valencia			Spain	Green	Spreading	Medium	Poor	85	48					
Jones's Square-head		W.	New York	do	do		V'y poor	90	74		June 1	Bald		
Jones's Winter Fife	C.	W.	do	do		Very lrg	Good	92			do	do	Velvet chaff	
Kastamuni	C.	W.	Turkey	Green	Nar. leaves	Medium	Fair	78	38	D	June 9	do		
Kathia	C.	W.	India	Lt. gr	Erect	do	V'y poor					Bearded		
Khel	C.	W.	do			Large		62			May 25	do		
King's Jubilee	C.	W.	Australia					57			May 14	Bald		June 21.
Kinney	C.		Oregon											
Kintama	C.	W.	Japan								May 30	Bearded		
Krasnokoloska	C.	S.	Russia	Lt. gr	Erect	Very lrg	Good		35	D. 3	June 16	Bald		
Kubanka	D.	S.	do	V'y. lt. gr	do	do	V'y good	35	(*a*)	D. 5	June 8	Bearded		
Kubb			Sweden					80	55			Bald		
Ladoga	C.	S.	Russia							D. 3				
Lal	C.		India					5			June 1	Bearded		
Lamed	C.	W.	Italy	Green	Spreading	Very lrg	Good	93		C. 3, D.	June 4	do		
Lancaster	C.	W.	United States					89		C. 3, D.	June 1	do		
Lehigh	C.	W.	do	Green	Erect	Very lrg	Good	83	63		June 4	do		
Linaza			Chile											
Little Club	Cl.	S.	United States					92		D. 3	May 23	Bald	Short, compact	
Lost Nation		W.	do					91			June 4	do	White chaff	
McKissick's Fife	C.	S.	do	Lt. gr				94	55	D. 3	do	do		

a Almost free.

Table presenting a comparative résumé of the principal qualities of 245 representative wheats of the world—Continued.

Name of variety.	Character of the grain.								District of United States to which best adapted.	Remarks.
	Size.	Color.	Hardness.	Physical qualities.		Per cent of nitrogenous matter.				
				Abs. wt. 100 gr.	Sp. gr.	Albuminoids.	Gluten.			
							Moist.	Dry.		
Herisson barbu	Small	Red	Semihard.			13.31	28.06	10.18	VII, VIII	
Herisson sans barbes	do	Amber	Soft			14.19	27.60	10.20	do	Plant with large, stiff straw.
Hickling			do			10.50	20.60	7.44	I, II	
Hopetown		White	do	*4.500		*13.30			I	* Sample from Colorado.
Hornblende	Medium		S. or semih			†13.18	†45.15	†13.09	III, VII	† Australian sample.
Hudson's Early Purple Straw	Medium	White	Soft			13.65	32.19	9.55	III, VIII	
Igel mit grannen			do			11.75	21.66	7.95	VII, VIII	
Igel ohne grannen			do						do	
Imperial	Small	Red	H. or semih			12.25	19.46	9.69	IV	Did well in Wisconsin.
Improved Fife	Medium	Dull white	S. or semih	 †3.672		*13.87 †14.18	*32.54 †45.12	*11.20 †16.16	II, III, VII	* Australian sample. † Sample from Colorado.
Japanese No. 1	do	Light br.	Soft	2.685	1.361				III, VIII	
Japanese No. 2	do	do	do						do	Escapes rust in Tennessee.
Japanese No. 4	do	do	do	2.850	1.388				do	
Jejar de Valencia						12.06	26.57	9.72		
Jones's Square-head			Soft	3.441		10.85	14.09	5.49	I VIII	
Jones's Winter Fife	Medium	Amber	do	*3.767 4.542	*1.371	 11.20	 20.55	 8.32	I, II	* Sample from Michigan.
Kastamuni			Semihard.			11.00	22.32	8.45	III, V, VI	
Kathia			Soft			9.16	16.74	6.47	I, II, III, VIII	
Khel			do						I, III, VIII	Lodged badly.
King's Jubilee	Medium	Dull white	do			9.25	1.50	0.69	I, III, VIII	
Kinney			do	5.368		8.93	17.09	6.69	VIII	
Kintama	Medium	Light br	do			16.13	34.58	12.40	III, VIII	
Krasnokoloska	Med. or sm	Red	H. or semih			12.44	27.09	9.64	IV	
Kubanka	Large	Yel'sh wh.	Vitreous			 †16.63 ‡11.19	*41.65 †37.79 ‡20.93	 †13.63 ‡8.50	V, VI, VII	* Sample from Caucasus. † Sample from Kursk. ‡ Sample from Germany.
Kubb			Soft			13.13	27.80	10.25	I, II	
Ladoga	Small	Red	Hard	4.118		15.23	30.59	12.17	IV	
Lal	Medium	Reddish				13.50	27.00	10.51	III, VIII	
Lamed	do	Red	Semihard.			*12.63 †11.56	*24.80 †25.15	*9.50 †9.02	II, V	* Sample from France. † Sample from Italy.
Lancaster	do	do	H. or semih						I, II, V	
Lehigh			Semihard.						do	
Linaza						10.69	23.28	8.50	VIII	
Little Club	Medium	Yel'sh wh.	Soft	4.225		10.15	21.61	8.32	VII, VIII	
Lost Nation	do	Red	do	*4.147		*12.08	*27.70	*10.12	I, II	* Sample from Colorado.
McKissick's Fife	Small	do	Hard						IV	

Table presenting a comparative résumé of the principal qualities of 245 representative wheats of the world—Continued.

Name of variety.	Group.	Spring or winter.	Where obtained.	Character of growing plant.					Hardiness.		Time of heading.	Character of the head.		Time of ripening.
				Autumn condition.				Spring condition	Resistance to leaf rust.	Resistance to cold or dr'ght.		Bald or bearded.	Other characteristics.	
				Color.	Manner of growth.	Amt. of growth.	Stand.							
Mealy	C.	W.	United States					91			May 27	Bald	Velvet chaff	
Medeah	D.	S.	Algeria					3		D.5	June 9	Bearded		
Mediterranean	C.	W.	United States					92	57	C.3,D.3	June 1	do		
Meekins	C.	W.	Russia					92		C.4,D.4	May 31	do		
Melka	C.	S.	Siberia	Green	Erect	Medium	Poor					do		
Mennonite	C.	W.	Russia	do	Sprdg. nar. lvs	do	Good	92	58	C.4,D.4	May 29	do		
Minnesota Fife	C.	S.	Minnesota			Large		80	23		May 25	Bald		
Mirado	P.	S.	Egypt (?)					50	(*a*)			Bearded	Velvet chaff	
Missogen	D.	S.	Greece					91	(*a*)		June 1	do		
Moscow	C.	W.	Russia					90	38		May 29	do		
Muzaffarnagar	C.	W.	India								June 1	do		
Mundia	C.	W.	do								do	(*b*)		
Murcia	D.	S.	Spain									Bearded		
Nab-el-bel	D.	S.	Algeria	V'y lt. gr	Erect, coarse	Medium	Fair							
Nashi	C.	W.	India	Green	Sprdg., very nar. leaves.	Large	V'y good	90	40	C.4,D.4	May 27	Bearded		
Nigger	C.	W.	United States	do	Spreading	Medium	Fair	91	59		May 25	do		June 23
Noé	C.		France					93	25		June 1	Bald		
Nonette de Lausanne	P.		do									Bearded		
Nonpareil	C.	W.	Australia					81			May 14	Mixed		
Odessa	C.	W.	Russia	Green	Spdg., nar. leaf	Large	Good	95	43		June 4	Bald		
Onigara	C.	W.	Japan	Yel. gr.		do	Fair	80	45		June 6	Bearded		
Oregon Club	C.	W.	United States	Green		do	V'y good	93	18		June 4	Bald	White chaff	
Palouse Blue Stem	C.	S.	Washington									do		
Penquites Velvet Chaff	C.	W.	do	Green	Spreading	Medium	Poor	91	53		June 4	Bearded	Velvet chaff	June 24
Petanielle noire de Nice	P.		France						(*a*)			do	do	
Pilli	C.	W.	India	Lt. gr.	Slender	Tall, l'rg		66			May 14	Bald		June 22
Pissi Hydrabadi	C.		do											
Polish	Pol.	S.	Russia	Lt. gr.	Erect			96	(*a*)	D.5	June 16	Bearded		
Poole	C.	W.	United States	Green	Spdg., nar. leaf	Large	Poor	91			June 1	Bald		
Power's Fife	C.	S.	North Dakota	Lt. gr.				91	53	D.3	do	do		
Pringle's Defiance	C.	W.	United States	Green	Erect, coarse	Very l.	Good	90	40		May 29	do		
Pringle's No. 5	C.	W.	do	do	Spdg., fine leaf	do	do	71	33	C.3,D.2	June 1	Bearded		June 23
Probsteier	C.	W.	Germany					70	29			Bald		
Prolifero	C.	W.	Italy	Dark gr.	Spdg., fine leaf	Large	Fair	90	40	C.3,D.3		Bearded		
Prophet	C.	W.	Persia					80	40		June 1	do		

a Almost free.

b Semibearded.

Table presenting a comparative résumé of the principal qualities of 245 representative wheats of the world—Continued.

Name of variety.	Character of the grain.								District of United States to which best adapted.	Remarks.
	Size.	Color.	Hardness.	Physical qualities.		Per cent of nitrogenous matter.				
				Abs. wt. 100 gr.	Sp. gr.	Albuminoids.	Gluten. Moist.	Gluten. Dry.		
Mealy	Medium		Soft						I, II	
Medeah	Large	Yel'sh wh.	Vitreous			*14.38 †9.63	*30.20 †12.75	*11.60 †5.16	V, VI, VII	*Sample from Germany. †Sample from N. S. Wales.
Mediterranean	Small	Red	H. or semih			*11.36	*35.10	*10.86	III, V, VI	*Australian sample.
Meekins	do	do	Hard	5.193		15.15			V, VI	
Melka	do	do	H. or semih			15.25	41.19	14.59	IV	
Mennonite	do	do	Hard						V, VI	
Minnesota Fife	do	do	do						IV	
Mirado	Large					12.66	25.05	9.21	VI, VIII	Plant short.
Missogen	do	Light yel.	Vitreous			16.63	37.92	14.22	V, VI	
Moscow	Medium	Red	Hard						do	
Muzaffarnagar	L. or med.								I, II, III	
Mundia			Soft			10.34	19.46	7.61	I, III	
Murcia	Large		H. or vit			12.06	23.63	9.27	VI	
Nab-el-bel	do		Vitreous						V, VI	
Nashi	Medium	Red	H. or semih						do	Lodged greatly. Plant very glaucous.
Nigger	do	Reddish	Soft	3.947		11.73	24.03	9.77	I, II	Lodged slightly.
Noé						12.44	27.83	9.81		
Nonette de Lausanne	Large								V, VI	
Nonpareil	Medium		Soft						III, VIII	
Odessa	Small	Red	Semih. or h						II, V	
Onigara	Medium	Light br	Soft			11.38	24.60	8.70	I, III, VIII	
Oregon Club	do	White	do	*3.647		*11.20	*23.75	*8.71	VIII	*Sample from Colorado.
Palouse Blue Stem			do	*4.572 *4.427		*13.48 *10.85	*33.76 *19.40	*12.02 *7.30	VII, VIII	*Samples from Washington.
Penquites Velvet Chaff	Medium	Red. or am	Semihard.						I, II	
Petanielle noire de Nice	Large		S. or semih			10.88	23.45	8.48	VI, VIII	
Pilli			Soft						III, VIII	Lodged slightly.
Pissi Hydrabadi				3.030	1.384	9.81	28.75	6.67	do	
Polish	Very large	Yel'sh wh.	Vitreous			†14.88	*46.67 †29.45	*15.66 †11.50	V, VI, VII, VIII.	*Australian sample. †Sample from Germany.
Poole	Medium	Amber	Soft	*4.030 †3.450		*14.00 †10.85	*32.44 †22.80	*12.48 †8.75	I, II	*Sample from West Virginia. †Sample from Indiana.
Power's Fife	Small	Red	Hard	2.683	1.375				IV	
Pringle's Defiance	Medium	White	Soft			14.93	33.16	10.62	II, VIII	
Pringle's No. 5	do		Semihard.			10.19	30.70	9.78	II, V	
Probsteier	do					8.25	17.22	6.53	I, II	
Prolifero	do	Red	Semihard.	3.815	1.395	12.25	24.91	9.31	II, V	
Prophet	do	Reddish	do			13.81	31.25	11.05	II	

Table presenting a comparative résumé of the principal qualities of 245 representative wheats of the world—Continued.

Name of variety.	Group.	Spring or winter.	Where obtained.	Character of growing plant.					Hardiness.		Time of heading.	Character of the head.		Time of ripening.
				Autumn condition.				Spring condition.	Resistance to leaf rust.	Resistance to cold or dr'ght.		Bald or bearded.	Other characteristics.	
				Color.	Manner of growth.	Amt. of growth.	Stand.							
Propo	C.		United States	Lt. gr				90	45		June 1	Bald	White chaff	
Pulavka	C.	W.	Russia	Green	Spreading	Large	Good	98	50		June 9	Bearded		
Purple Straw	C.	W.	Australia	do	Erect, nar. leaf	Very lrg	do	91	63		June 4	Bald		
Quartz	C.	W.	Colorado	do	Spreading	Medium	Poor	91	55		May 29	Bearded		
Rattling Jack	C.	W.	Australia	do		Very sm	V'y poor	68	30		May 31	Bald		
Red Bearded	C.	W.	Russia	do	Spreading	Medium	Fair		57	C.3, D.3		Bearded		
Reddish White Bearded	C.	W.	Persia	do		do	do	65	40	C. D	June 1	do		
Red Fife	C.	S.	Canada	Lt. gr				91	50	D. 3	do	Bald		
Red Provence	C.		France	Green	Erect, nar. leaf	Large	Fair	90	68		do	do		
Red Spring	Em.	S.	Germany						(*a*)			Bearded		
Red Tyrol	S.		do						33			Bald		
Red Winter	C.	W.	Russia	Green	Spdg., fine leaf	Medium	Poor		58	C.4, D.4	June 7	Bald		
Rice	C.	W.	United States		Broad leaves			91			May 27	do		June 21
Rieti	C.	W.	Italy	Green	Spreading	Medium	Fair	76	28	C.3, D.3	May 31	Bearded		
Rio Grande	C.	S.	United States	do		Small	V'y poor	91			do	do		
Roseworthy	C.	W.	Australia	Lt. gr	Erect	Large	Poor	90	38		May 10	(*b*)		June 20
Rudy	C.	W.	Ohio	Green	Spreading	do	V'y good	89	67		June 1	Bearded		
Russian Hard	C.	W.	Russia	do	do	do	do		53	C.4, D.4		do		
Russian Spring	C.	S.	do					89		D. 4				
Rye Wheat	C.	W.	Germany					65	48		June 7	Bald	Velvet chaff	
Safeed	C.		Persia	Green		Medium	Poor			Cold		Bearded		
Saida			Egypt								May 25	do		
Saldomé	C.	W.	Argentina	Lt. gr	Erect	Large	Good			D		do		
Samara	C.		Russia		Erect, nar. leaf			90	(*a*)	D. 5	June 1	do		June 23
Sandomir	C.	W.	do	Green	Spreading	Small	Poor		30		do	Bald		
Saratov	C.		do	Lead	Erect, nar. leaf			94		D. 5	June 4	Bearded		
Sarui-bug-dai	D.	S.	Turkestan							do				
Saskatchewan Fife	C.	S.	Canada					94	63	D. 3	June 4	Bald		
Saumur Winter	C.	W.	France											
Scotch Fife	C.	S.	Canada							D. 3				
Seneca Chief	C.	W.	United States	Dark gr.	Erect	Very lrg	V'y good	91	79		June 4	Bearded		
Seven-headed	P.			Lt. gr	Erect, velvety, coarse.	Medium	Fair	94			June 1	do		June 23
Shirosawa	C.	W.	Japan					80			May 30	do		
Shiro-yemidashi	C.	W.	do					78			May 27	do		
Sicilian Red Square-head	Cl.		France						(*c*)		June 9	Bald	Sq. shaped	

a Almost free.

b Semibearded.

c Rather rust resistant.

Table presenting a comparative résumé of the principal qualities of 245 representative wheats of the world—Continued.

Name of variety	Character of the grain.								District of United States to which best adapted.	Remarks.
	Size.	Color.	Hardness.	Physical qualities.		Per cent of nitrogenous matter.				
				Abs. wt. 100 gr.	Sp. gr.	Albuminoids.	Gluten.			
							Moist.	Dry.		
Propo	Medium	White	Soft						VII, VIII	
Pulavka	do	Light yel.	do	4.205	1.354	11.88	28.30	10.43	I, VII, VIII	
Purple Straw	do	Dull white	do	5.478		8.58	18.72	7.00	III, VIII	
Quartz	do		do						III, VII, VIII	
Rattling Jack	do	White	do			10.57	29.56	8.93	VII, VIII	
Red Bearded	Small	Red	Hard	3.500	1.394				V, VI	Yield in Kansas 19¼ bu. per acre. Did well in Michigan.
Reddish White Bearded	Medium	Reddish	Semihard.	3.750	1.338	14.88	33.72	12.13	VI	
Red Fife	Small	Red	Hard	*4.105		*15.84	*37.11	*14.40	IV	* Sample from Canada.
Red Provence									I, II	
Red Spring			H. or semih						IV	
Red Tyrol									II, V	
Red Winter	Small	Red	Hard			14.19	36.38	12.77	do	Yield in Kansas, 16¾ bu. per acre; in Colorado, 27½ bu. per acre.
Rice									I, II, III	Slightly lodged.
Rieti	Med. or l.	Reddish	Semihard.			13.81	27.02	9.76	II, V	
Rio Grande	Medium	do	H. or semih	*3.276 †4.743		*11.38 †12.43	*22.25 †32.83	*9.14 †11.76	IV	* Sample from Canada. † Sample from Colorado.
Roseworthy	do	*	Soft						III, VIII	* White or light brown.
Rudy	do	Amber	Semihard.						II	
Russian Hard	Sm. or med.	Red	Hard						V	
Russian Spring	Small	do	do						IV	
Rye Wheat			Soft			*14.50 †11.88	*34.99 †23.76	*12.25 †9.20	II	* Sample from Germany. † Sample from France.
Safeed			Semihard.						II, VI	
Saida									VI	Plant very short.
Saldomé	Large	Yel'sh wh	Semihard.						do	
Samara	Small	Red	Hard						V, VI	Lodged slightly.
Sandomir	Med. or sm.	White	Soft			*10.31	*20.94	*7.94	I, II, III	Lodged considerably. * Sample from Germany.
Saratov	Small	Red	Hard						V, VI	Plant velvety hairy.
Sarui-bug-dai	Large	Yel'sh wh	Vitreous						V, VI, VII, VIII.	
Saskatchewan Fife	Small	Red	Hard	3.720		14.35	30.80	12.10	IV	Sample from Wyoming.
Saumur Winter									II	
Scotch Fife	Small	Red	Hard	*1.617 †2.818		*8.75 †17.15	*12.96 †39.05	*5.13 †14.65	IV	* Sample from Oregon. † Sample from Nebraska.
Seneca Chief	Medium	Reddish	Semihard.						II	
Seven-headed									VI, VII	Lodged slightly.
Shirosawa	Medium	Light br.	Soft			10.00	20.39	7.88	III, VIII	
Shiro-yemidashi	do	do	do			16.44	37.40	13.42	do	
Sicilian Red Square-head	do	Dull red'sh	Semihard.			*15.18 10.50	*45.74 15.70	*13.98 6.30	VIII	* Australian sample.

Table presenting a comparative résumé of the principal qualities of 245 representative wheats of the world—Continued.

Name of variety.	Group.	Spring or winter.	Where obtained.	Character of growing plant.					Hardiness.		Time of heading.	Character of the head.		Time of ripening.
				Autumn condition.				Spring condition.	Resistance to leaf rust.	Resistance to cold or dr'ght.		Bald or bearded.	Other characteristics.	
				Color.	Manner of growth.	Amount of grow h	Stand.							
Sindhi			India								June 1	Bearded		
Sonora	C.	W.	Mexico	V'y lt.gr	Erect	Large		87		D. 3	May 23	Bald	Velvet chaff	
Soules	C.	W.	United States					91	72		June 1	do		
Spring Ghirka	C.	S.	Russia	Gray gr	Erect, velvety	Large	Fair	97	36	D. 4	June 11	do		
Steinwedel	C.	W.	Australia	Lt. gr				71			May 27	do		June 24
Swamp	C.	W.	United States					91			June 1	Bearded		
Taganrog	D.	S.	Russia		Erect, nar. lvs.			90	5	D. 5	June 4	do		
Talavera	C.	W.	Australia					85			June 7	Brd'd (?)		
Tangarotto	C.	W.	Italy	Green	Spreading	Medium	Poor		(*a*)	C D	May 27	Bearded		
Taos	C.	W	New Mexico											
Tasmanian Red	C.	W.		Green	Spreading	Medium	Fair	91	54		May 27	Bearded		Do.
Theiss	C.	W.	Hungary	do	do	Very sm	V'y poor	89	50		June 6	do		
Tourmaline	C.	W.	Colorado		Erect, nar. lvs.			75	67		June 7	Bald	White chaff	
Touzelle	C.		France					90	44			Brd'd (?)		
Trimenia	C.	W.	Italy					5	(*a*)	D	June 9	Bearded		
Turkey	C.	W.	Russia					91		C.4, D.4	June 1	do		
Tuscan	C.	W.	Australia		Rather short			92			May 23	Bald		June 23
Urtoba	C.	W.	Germany						36			do		
Valley	C.	W.	United States					92		C. 2	May 27	Bearded		June 24
Varesotto	C.	W.	Italy	Green		Small	V'y poor	93	65	C. 3	do	do		
Velvet Blue Stem	C.	S.	United States							D. 3		Bald	Velvet chaff	
Velvet Chaff	C.	W.	do	Green	Spreading	Medium	Good	93			May 9	Bearded	do	
Victoria d'Automne	C.	W.	France						40		June 23	Bald		
Victorian Defiance	C.	W.	Australia					92	62		May 27	do		
Volo	D.	S.	Greece	Lt. gr	Erect	Medium	Fair			D	do	Bearded		Do.
Vyssoko-Litovsk	C.	W.	Russia						58		June 7	Bald		Do.
Walla Walla			United States			Large		30			May 25	Bearded		
Walker	C.	W.	do	Dark gr.	Spr'ding nar. leaves.	Medium	Fair	93	58		May 31	Bald		June 23
Ward's Prolific	C.	W.	Australia	Lt. gr	Spreading	Large	Fair	58	50		June 1	do	Reddish chaff	Do.

a Almost free.

Table presenting a comparative résumé of the principal qualities of 245 representative wheats of the world—Continued.

Name of variety.	Character of the grain.								District of United States to which best adapted.	Remarks.
	Size.	Color.	Hardness.	Physical qualities.		Per cent of nitrogenous matter.				
				Abs. wt. 100 gr.	Sp. gr.	Albuminoids.	Gluten.			
							Moist.	Dry.		
Sindhi				3.315	1.386	14.38	28.52	11.11	VIII	
Sonora	Medium	Yel'sh wh	Soft	*3.830		*12.25	*36.18	*13.37	VII, VIII	Short growth. *Sample from Colorado.
Soules			do						II	
Spring Ghirka	Small	Red	Hard			*15.75	*41.23	*14.98	IV	*Sample from Samara.
Steinwedel			Soft	5.723		9.63	21.90	8.11	III, VIII	
Swamp			Semihard	2.795		14.	19.27	11.55	II	Lodged slightly.
Taganrog	Large	Yel'sh wh	Vitreous			15.06	32.75	12.15	V, VI, VII, VIII	
Talavera			Soft						I, II	
Tangarotto	Medium	Reddish	Semihard			14.19	30.65	12.24	II, III	
Taos	do	White	Soft	4.686		9.45	21.50	7.80	VII	
Tasmanian Red	do	Red	Semihard						II, III	Do.
Theiss	do	do	Hard	 3.535	 1.414	*15.38 †12.49	*38.16 †26.90	*12.87 †10.12	do	Yield in Kansas 17¼ bu. per acre; Colorado 33 bu. *Sample from Kursk. †Sample from Russia. Weight per bu. in Colorado 48 lbs.
Tourmaline	Med. or sm	White	Soft	3.058	1.383				III, VII, VIII	
Tonzelle			do	*4.300 		*14.18 11.56	*44.64 26.65	*14.88 9.25	I, II	*Sample from Colorado.
Trimenia			Vitreous			*12.63 †11.88	*24.64 †24.04	*9.68 †9.30	V, VI, VIII	*Sample from Germany. †Sample from Italy.
Turkey	Small	Red	Hard	*2.820 3.128	 1.334	 12.43	 32.80	 12.25	V, VI	*Sample from Kansas.
Tuscan			Soft						VIII	
Urtoba			do			15.56	32.95	11.95	II	
Valley	Medium	Reddish	Semihard						do	
Varesotto	do	do	do			11.75	25.00	9.08	II, V	
Velvet Blue Stem	Small	Red	Hard	*3.485 †2.559		*15.49 †15.23	*35.71 †33.68	*13.69 †13.08	IV	*Sample from South Dakota. †Sample from Nebraska.
Velvet Chaff		Reddish	Semihard						II	
Victoria d'Automne			Soft			10.69	15.55	6.32	I, II	
Victorian Defiance			do	4.435	1.384				I, III, VIII	
Volo	Large	Amber yel	Vitreous			*12.06 †15.25	*28.00 †34.05	*10.22 †12.45	V, VI, VII, VIII	Lodged slightly. *Sample from Greece. †Sample from Germany.
Vyssoko-Litovsk	Medium	Light yel	Soft			11.88	28.76	10.35	I, VII, VIII	Yield in Kansas 19 bu. per acre.
Walla Walla			do						VII, VIII	
Walker	Medium	Reddish	Semihard						I, II	
Ward's Prolific	do	Amber	Soft	4.185	1.368				I, III, VIII	

Table presenting a comparative résumé of the principal qualities of 245 representative wheats of the world—Continued.

Name of variety.	Group.	Spring or winter.	Where obtained.	Character of growing plant.					Hardiness.		Time of heading.	Character of the head.		Time of ripening.
				Autumn condition.				Spring condition.	Resistance to leaf rust.	Resistance to cold or dr'ght.		Bald or bearded.	Other characteristics.	
				Color.	Manner of growth.	Amount of growth	Stand.							
Wellman's Fife	C.	S.	United States									Bald		
White Tuscan	C.	W.	Australia	Green	Erect	Large	Poor	25	35		June 8	do		
White Winter	C.	W.	Oregon											
Wild Goose	D.	S.	United States					89	(*a*)	D. 4	June 4	Bearded		
Winter Ghirka	C.	W.	Russia	Dark gr.	Spr'ding fine leaves.	Medium	Good		58	C.4, D.4	June 17	Bald		
Wyandotte Red	C.	W.	United States					93			May 31	do		
Yemide	C.	W.	Japan	Yel. gr	Erect, coarse	V'y large	V'y good	60	40		May 27	Bearded		
Yx	C.	W.	Russia	Green		Large	Fair		63	C.4, D.4		do		
Zaruta	C.	W.	Japan					50			June 1	do		
Zimmerman	C.	W.	United States	Lt. gr	Erect, nar. lvs	Large	V'y good	93	72	Cold 3	May 24	Bald		June 21

Name of variety.	Character of the grain.								District of United States to which best adapted.	Remarks.
	Size.	Color.	Hardness.	Physical qualities.		Per cent of nitrogenous matter.				
				Abs. wt. 100 gr.	Sp. gr.	Albuminoids.	Gluten.			
							Moist.	Dry.		
Wellman's Fife	Medium	Red	Hard	2.628	1.347				IV	
White Tuscan			Soft	*5.415 		*8.75 11.19	*20.94 22.36	*8.33 8.94	I, III, VII, VIII	*Sample from Australia.
White Winter	Medium	Yel'sh wh	do	*4.724 *4.898		*8.75 *9.63	*14.53 *12.33	*5.58 *4.70	VIII	*Both samples from Oregon.
Wild Goose	Large	Yel'sh br	Hard or vit	3.697 *4.655	1.379 	 *13.48	 *32.56	 *13.09	IV, V, VI, VIII	*Sample from Canada.
Winter Ghirka	Small	Red	Hard	2.415	1.408	*13.63	*33.79	*12.23	II, V	*Sample from Kharkov. Yield in Kansas 18 bu. per acre; Colorado 25½ bu. Weight in Colorado 60 lbs. per bu.
Wyandotte Red	Medium	Reddish	Semihard						II	
Yemide	do	Light br	Soft			11.19	20.37	7.77	III, VIII	
Yx	do	Red	Hard			15 75	41.05	13.82	II, V	Yield in Kansas 21 bu. per acre; Colorado 26⅔ bu. Weight in Colorado 61 lbs. per bu.
Zaruta	do	Light br	Soft			10.13	20.41	7.70	III, VIII	
Zimmerman	do		Semih.or s						I, II, III, V	Lodged badly.

a Almost free.

In comparing the value of different varieties it is very desirable to know both the absolute weight and specific gravity of the grain, as these physical qualities bear some relation to the chemical composition of the grain and to the nature of the plant in general. All the information concerning specific gravity, as well as a number of determinations of absolute weight given in the table, are the results of a series of interesting investigations made in the Seed Laboratory of the Division of Botany of this Department by the late Mr. J. C. Dabney, then a member of that Division. Almost all the data of the table concerning nitrogen content of the grain are the result of chemical analyses made under the direction of Dr. H. W. Wiley, chief of the Division of Chemistry. The greater part of these analyses were made at the request of the chief of this Division with samples furnished by the Division. The remainder, together with a number of determinations of absolute weight, are taken from reports of work formerly done by the Division of Chemistry.[1] A few analyses are also given on the authority of F. B. Guthrie[2] and Emerich Pekar.[3]

As the value of the grain for making bread and macaroni is measured almost wholly by its quality and quantity of gluten content, only the percentage of moist and dry gluten and the total per cent of albuminoids are given.

It will be noted that no column for yields is given in the table. For this omission, which under other circumstances would be a serious one, there are three good reasons: (1) In experiments of this kind each variety is given so small a space (an average space of one drill row 35 feet long) that it is not practicable to obtain accurate estimates of yield. (2) Many of the varieties tested are already well-known American wheats, whose yields have often been reported by various experiment stations, while as to the foreign sorts it is most important, first of all, to know whether they will prove to be suited to our conditions at all or not, before we are ready to test their yielding capacity. (3) The yield of a variety, while it is of course directly the biggest thing to the practical man, is, after all, not a distinct constant quality in itself, but is the combined result of a number of qualities acting indirectly, and often not thought of at all. For example, such sorts as Clawson and Poole are fairly good wheats, and under mild conditions would probably yield better than Turkey; but in west Kansas or southern

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[1] See "Analyses of Cereals collected at the World's Columbian Exposition," Bul. No. 45, Div. Chem., U. S. Dept. Agric., pp. 39–53, 1895.

[2] "Notes on the milling qualities of different varieties of wheat," Dept. Agric. N. S. W., misc. pub. No. 189, p. 47, 1898; "Milling notes on the Lambrigg harvest of 1897–98," Agric. Gaz. N. S. W., Vol. X, Pt. 9, pp. 906–915, Sept., 1899, and "Absorption of water by the gluten of different wheats," Dept. Agric. N. S. W., misc. pub. No. 104, p. 7, 1896.

[3] "Weizen und Mehl unserer Erde," im Auftrage des Klg. Ung. Ministeriums für Ackerbauindustrie und Handel, pp. 277, Budapest, 1882.

Nebraska they would fail entirely in certain seasons because of drought or cold, while Turkey, being very hardy, would produce a much larger yield on an average than either of the former, though its absolute yield in a good season might not be so great. So, also, it is found in the Palouse country that there are certain varieties which have absolute yields in that region greater than those of the Little Club or Palouse Blue Stem, but they shatter so badly that the net yield of the latter is greater.

As regards the field trial experiments upon which is based the larger part of the results given in the table, it must be said that many of those sorts whose behavior indicated that they would not be well adapted for our use should be further tested before adverse judgment is pronounced upon them, especially so if their qualities in other respects are good. Nevertheless, the table as a whole shows pretty accurately which are the best varieties for different districts of the country.

Nothing can be more interesting than the constant observation from year to year of the efforts being made by varieties from every country in the world, struggling with new conditions of soil and climate, to obtain a footing in a strange land. The gradual elimination of the less-adapted sorts by the severity of winter, drought, etc., soon shows unmistakably which are the varieties that will be most valuable. Of course it may truthfully be objected that mere hardiness is not of value by itself if other qualities are not also present. But, on the other hand, it is a further matter of interest how different qualities are often so closely associated in the same varieties that if a variety is adapted to a certain district with respect to one quality, it is apt to be so with respect to at least one or two other equally valuable qualities, though, of course, there are serious exceptions. It is also quite worthy of note that some apparently insignificant characteristics bear an important relation to the presence of qualities of direct economic importance. As an example of these we may note especially the characteristics of the young plant in its autumn stages in connection with the presence of certain economic qualities. Hardy winter varieties are rather slow starting in the fall, but produce good roots and soon spread out flat on the ground in preparation for the cold and snow of winter. The leaves are narrow and usually dark green or purplish at first, especially near the roots. Spring varieties and most durums and poulards, as well as some of the weaker winter sorts, on the other hand, germinate quickly and make a large growth in the autumn, but are cut short or entirely killed by the severity of the winter. They produce coarse, light-green leaves, but poor roots. In regions of mild winters durum and poulard wheats make excellent pasturage because of their rapid autumn growth. There is really very little, if any, check to the growth from seeding till harvest in localities well adapted for these varieties.

One well acquainted with wheat varieties is usually able to determine largely their general classification simply from their appearance in the autumn.

It will be seen also by a study of the table that there is a very close constant relation between hardiness, color, size, weight, and hardness of grain, and chemical composition in varieties of the common group. Varieties very resistant to cold and drought have small, hard, red grains, possessing a large proportion of albuminoids and a relatively high specific gravity, though the absolute weight is likely to be low. It is also a general rule that bearded varieties are less susceptible to leaf rust, but there are a number of important exceptions to this rule. Varieties with harsh, hairy, or glaucous leaves are also usually rather resistant to this rust. Varieties early in ripening are often dwarfed, and come from warm regions nearly always. Hard-grained winter varieties are bearded, as a rule. Drought resistant sorts, whether bald or bearded, white or red-grained, possess a larger proportion of nitrogen than those which succumb to drought.

The effect of a change of environment upon the wheat plant has already been referred to. That marked changes are effected in this way is proved, with respect to chemical composition, by the facts given in the table. In a number of instances duplicate analyses are given of samples of the same variety obtained from different localities. Almost invariably the samples from hot and more or less arid districts show a larger per cent of gluten content. In some instances the difference is considerable. Alsace wheat from Ekaterinoslav (Russia) furnishes 13.58 per cent of dry gluten, while the same variety from Poltava, farther northwest in a moister region, shows only 9.30 per cent. Improved Fife, though a much liked variety in Australia, produces but 11.20 per cent of gluten there in comparison with 16.16 per cent in Colorado. Kubanka from Kursk (Russia) possesses 37.79 per cent moist gluten and 13.63 per cent of dry gluten, while as grown in Germany it furnishes only 20.93 per cent of moist gluten and 8.50 per cent of dry gluten. At the same time a sample from the Caucasus shows 41.65 per cent of moist gluten. A remarkable difference is shown in the case of Scotch Fife from Nebraska and Oregon. The former sample contained 14.65 per cent of dry gluten, while the latter contained only 5.13 per cent, slightly over one-third as much. There is a striking example in the case of Palouse Blue Stem of a difference in gluten content between two samples from the same State, Washington. These samples, however, were no doubt from different localities, and no two regions are likely to be much more different from each other than are western Washington and the Palouse country of eastern Washington.

A most interesting example of correspondence between climate and chemical composition of the grain is exhibited in the case of samples from Kursk. As will be seen in the table, all samples from this locality not only show a very large per cent of gluten, but also a per cent always far above that of other samples of the same varieties from other localities. The Kursk samples are uniformly so superior in this respect that one naturally looks about for an explanation. The matter is no doubt to be explained in this way: It is a fact already discussed by the writer in another publication[1] of this Department and referred to before in this bulletin that the nitrogen content of the grain is greatest in regions having black soils, extremes of temperature, and very low rainfall. In Russia extreme heat and aridity increase eastward and southward as a rule. The government of Kursk, however, presents a remarkable exception to this rule, especially as regards rainfall. The normal yearly rainfall is 16.9 inches, while in Woronetz, Tambov, and Ekaterinoslav, east and south of it, the normal is 21 and 22 inches. It is apparently situated in an arid area, with greater rainfall all around it. At the same time the extremes of temperature are great and the soil is of the best in the "Chernozem" (black earth) region.

As before stated, all the experiments and observations which form the basis of this table have been made with the view of obtaining some reliable foundation for future wheat improvement. The general conclusions of immediate value to the wheat growers that are to be drawn from this work of the Department, and which are of rather wide application, may be stated as follows:

(1) Considering all qualities, the best wheats in the world are of Russian origin, coming particularly from eastern and southern Russia. They are resistant to cold and drought, are more or less resistant to leaf rust, and have the best quality of grain. They are fairly early in ripening and are good yielders. Under the head of remarks the yields per acre of several newly introduced Russian sorts in Kansas and Colorado are given in the table. For varieties not yet acclimated it will be seen that these yields are very good. The yields and weights per bushel in Colorado are furnished by W. F. Crawley, superintendent of the Arkansas Valley Experiment Station at Rockyford in 1897. The following may be considered as the best Russian varieties so far known: Arnautka, Kubanka, Kubanka Red Winter, Crimean, Sandomir, Ulka, Chernokoloska, Buivola, Red Winter, Bearded Winter, Yx, Odessa, Sarui-bug-dai, Ghirka Spring, Ghirka Winter, Russian, Beloturka, Mennonite, and Turkey. (See Plate VIII, Fig. 1.)

(2) The earliest ripening wheats are often dwarfed and come principally from India, Australia, and Japan, though a few are from the Mediterranean region. They are usually soft white wheats, but those from Japan are red, rather hardy, and possess a fair gluten content.

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[1] Russian cereals adapted for cultivation in the United States, Bul. No. 23, Div. Bot., pp. 8–11.

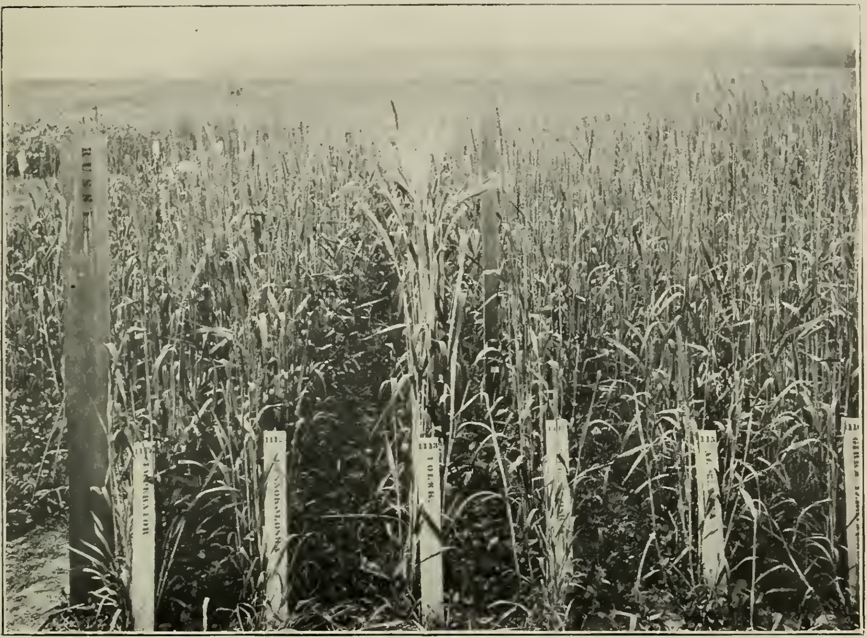

FIG. 1.—GROUP OF RUSSIAN WHEATS IN EXPERIMENTAL PLATS AT GARRETT PARK, MD. (ORIGINAL.)

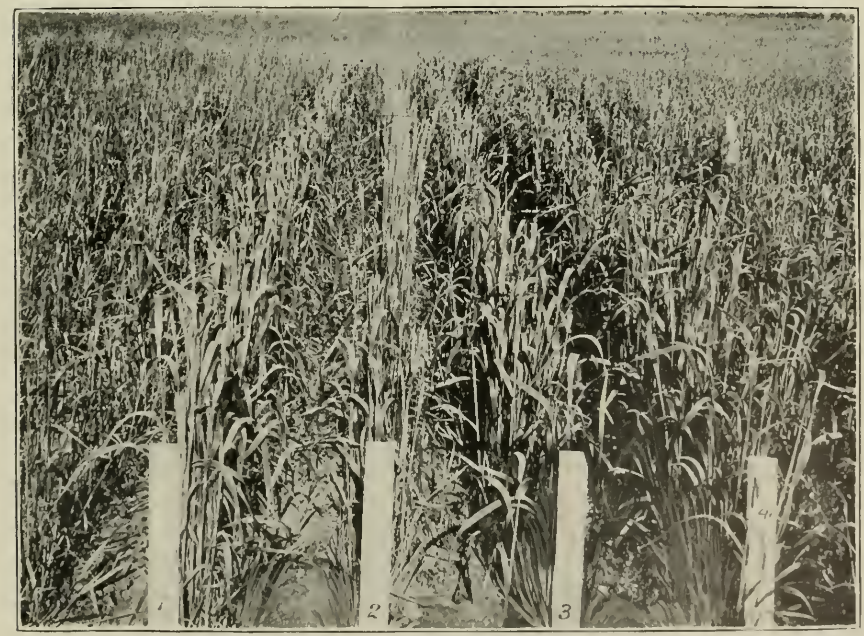

FIG. 2.—EXPERIMENTAL WHEAT PLATS AT GARRETT PARK, MD., SHOWING EARLINESS OF KING'S JUBILEE; 1, LEAKS; 2, KING'S JUBILEE; 3, TUSCAN; 4, PURPLE. (ORIGINAL.)

The best varieties so far known for our use from these regions are: Early Japanese, Yemide, Kintama, Japanese No. 2, Onigara, Daruma, Japanese No. 1, Japanese No. 4, Shiro-yemidashi, Allora Spring, Steinwedel, Early Baart, King's Jubilee (Plate VIII, Fig. 2), Roseworthy, Canning Downs, Kathia, and Nashi.

(3) Though varieties of Russian origin are, on the whole, the best, there are certain sorts from other countries which behave much like them. These are Fulcaster, Lancaster, Tasmanian Red, Fultz, Chubut, Prolifero, Rieti, Nashi, Mediterranean, Tangarotto, and Valley.

(4) Durum, Polish, and poulard wheats, besides being admirably adapted for making macaroni, are all rather resistant to leaf rust. The best known varieties are: Arnautka, Kubanka, Beloturka, Medeah, El Safra, Galland's Hybrid, Petanielle noire de Nice, Chernokoloska, Sarui-bug-dai, Volo, Missogen, Atalanti, Cretan, Wild Goose, Polish, and Nicaragua.

(5) Common bread wheats can not be depended upon to resist rust, but the best in this regard are: Turkey, Crimean, Pringle's Defiance, Rieti, Oregon Club, Fulcaster, Odessa, Pringle's No. 5, Mennonite, Velvet Blue Stem, Saskatchewan Fife, Mediterranean, Alsace, Nashi, Ghirka Spring, Prolifero, Bellevue Talavera, Ghirka Winter, Red Winter, Bearded Winter, Theiss, Deitz Longberry, Arnold's Hybrid, Sonora, and Banat.

(6) Einkorn resists leaf rust completely, and emmers resists it to a high degree at least.

(7) The very hardiest winter varieties are Turkey, Crimean, Red Winter, Ghirka Winter, Yx, and Bearded Winter. During the unusually severe winter at Manhattan, Kans., in 1896–97, these varieties fared very well when nearly all the experimental varieties of the regular experiment station plats at that place winterkilled, though well acclimated.

(8) Club wheats are usually soft grained and tender sorts and adapted only to mild climates, like that of California. They are excellent yielders. Among the best of them are: Little Club, California Club, Palouse Red Chaff, Sicilian Red Square-head, Herisson barbu, Herisson sans barbes, and Chili Club.

WHEAT BREEDING.

If we wish to continue our improvements in wheat culture, it is evident that we must soon depend upon other means than simply the introduction of varieties new to the country. During the earlier history of the country it was a question even whether wheat could be grown at all in many of the new regions open to settlement, and practically every variety had to be tested. Their introduction, therefore, naturally played the greater part in wheat improvement, and has continued to do so, in less measure of course, almost to the present time. But the time will soon arrive when there will be no further varieties to introduce better than we already have. The work now being done by the Section of Seed and Plant Introduction of the Division of Botany of this Department is especially hastening the approach of this period. So far as our knowledge goes at present, there are now but two regions in the world which produce varieties likely to be of particular value to this country from which we have not already secured seed for trial in considerable amounts. These regions are (1) the northern portions of India and China, including Tibet, and (2) Abyssinia. There are still some of the very best varieties to be obtained, however, from regions already drawn upon, such as southeast Russia, Turkestan, and Japan. No more important work could be done at present than that of securing all these new sorts from different regions, for of course it is a great waste of time and labor to the wheat breeder to spend years in the production of varieties having special qualities if other sorts already possessing these qualities can be readily obtained from other countries.

But, as stated at the beginning of this report, although many valuable improvements have resulted and are likely still to result from introduction, there are often certain combinations of qualities found to be extremely desirable for a particular region which, so far as we yet know, do not exist in any one variety, native or introduced. Such ideal sorts are therefore to be acquired by improvements of the varieties now in use, which must be accomplished through hybridization and selection. Besides, in certain varieties ideal in other respects, such qualities as rust resistance, yielding capacity, etc., may exist already, but not to a sufficient degree. In such cases these qualities must be increased by selection of seed from individuals which exhibit them to the greatest degree. But manifestly the greater number of varieties one has at hand, either native or introduced, especially if these have been chosen with great care, the greater are the number of chances offered him for selecting and improving these qualities. The trial of introduced sorts, therefore, in comparison with native ones simply gives one a practical knowledge of the facts herein discussed under the heading "Sources for desirable qualities." With these facts in mind, together with those concerning characteristics and needs of the different wheat districts, one is prepared for effective work in wheat improvement.

IMPROVEMENT BY SELECTION.

During the last thirty or forty years considerable work has been done in wheat breeding through selection, though it is only a beginning in comparison with the great amount that may be done. It may be of interest to note a few of the most important instances of the actual production of new sorts in this way.

In 1862, in Mifflin County, Pa., Abraham Fultz, while passing through a field of Lancaster wheat, which is a bearded variety, found three heads of bald wheat. He sowed the seed from these heads the same year, and continued sowing a larger amount each year, until he obtained sufficient seed to distribute it pretty well over the country. It soon became a well-marked and popular variety, called Fultz from the name of the breeder, and is now the best known of American wheats. In 1871 this Department distributed 200 bushels of the wheat for seed. This variety is rather early in ripening, fairly hardy, and possesses a semihard, red grain of good quality. It comes nearest being a general purpose wheat of all our varieties, being grown with good success in nearly all parts of the country and in several foreign countries.

Next to Fultz, one of the best known of our native wheats is White Clawson, or simply Clawson. This variety originated in Seneca County, N. Y., in 1865, through the selection of certain superior heads from a field of Fultz by Garrett Clawson. On planting the grain from these heads, both a white and red-grained sort resulted the following season. The white wheat was considered the best, and the pint of seed obtained of this sort was sown, producing 39 pounds the following season. The third year after this 254 bushels were harvested, and that season the variety was distributed to other farmers. In 1871 this variety took first premium at the Seneca County fair, and in 1874 seed was distributed by this Department. Though judged inferior by millers at times, this variety has become a very popular one. It must not be confused with Early Red Clawson, a very distinct variety. It is a bald wheat, rather hardy, with soft, white, or light amber grains. Early Red Clawson, because of its earliness, has taken the place of this variety to a great extent in recent years.

One of the best of the more recently produced varieties is the Rudy, which was originated at Troy, Ohio, in 1871, by M. Rudy, through a careful propagation of the seed from a superior and distinct stool of wheat found in a large field. It is a semihard or soft reddish-grained wheat, bearded and with white chaff. It is widely grown in Ohio, Indiana, and adjoining States.

A number of tne different varieties of Fife and Velvet Blue Stem of the spring-wheat States were also produced by simple selection. Wellman's Fife is a good example. In 1878 D. L. Wellman, of Frazee City, Minn., received a sample package of Scotch Fife wheat from the Saskatchewan Valley, in Manitoba. This was sown in the spring of the following year, and as a result it was found that the seed was badly mixed. Removing all plants but those of the true Fife and propagating carefully from year to year, Mr. Wellman gradually bred upward a very pure strain of the Fife, which became known as the Saskatchewan Fife. From the crop of 1881 were selected some unusually large heads, and from the seed of these as a beginning he finally produced a rather distinct sort, now known as Wellman's Fife. In a similar manner Powers's Fife, Hayne's Blue Stem, Bolton's Blue Stem, and other sorts have been produced by the men whose names they bear.

By the process of selection an unusually good variety of white wheat for the Eastern States, usually called Gold Coin, has very recently been produced by Ira W. Green at Avon, N. Y. Several years ago he grew a field of Diehl Mediterranean, a bearded, red-grained wheat, and while passing through this field one day found a bald head possessing white grains. Planting every grain of this head, he found as a result next season that he had heads with very long beards, some with short beards, and others with none at all. The grain also was mixed, some red and some white. He desired a bald wheat, since the beards interfered with his success in woolgrowing, hence only the grains from the bald heads were again planted. From this as a beginning, a practically new variety resulted, which he called "No. 6." It has proved to be of considerable value for certain localities, and is already pretty well known. Various names have been given to it by different seedsmen, but it is best known by the name Gold Coin.

In instances like those just related the change has been so great as to produce really a new variety. But, of course, the majority of improvements made by selection do not represent such marked changes, though there is a great tendency among breeders to establish new varieties on the basis of very slight improvements. In a majority of the instances above described the circumstances too are such that one can not escape the thought that the abnormal heads found in the fields were the result of natural crosses. In fact in the cases of Clawson and Gold Coin wheats this is almost certain, since the seed from the first heads continued to produce sporting progeny, the following year. Or it is possible in the case of Gold Coin that the sporting was simply a later cropping out of this phenomena in the Diehl Mediterranean, which is itself a hybrid. Besides these cases, there are also instances mentioned by other writers which pretty well establish the fact of the occurrence of natural crosses among wheat varieties,[1] though, of course, such occurrences are rather rare. On the other hand, in the work of hybridization the selection of parent forms and the after selection of the best individuals from the sporting offspring are by far the most critical operations to be performed. Hence selection is both the most important part of all the work of wheat breeding, and is also to be considered from two rather different standpoints: (1) that of its operations in connection with hybridization (natural or artificial), and (2) in making the ordinary less striking improvements in the same variety. The former phase will be best discussed under the subject of hybridization.

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[1] See especially Rimpau's statements in his article on "Kreuzungsprodukte landwirthschaftlicher Kulturpflanzen," in Landwirthschaftliche Jahrbücher, Bd. xx, S. 347–350, 1891.

In cases like those of the different varieties of Fife and Velvet Blue Stem, such as Wellman's Fife, Hayne's Blue Stem, etc., above mentioned, as well as many others that might be described, the new sort, if it is rightly called such, has been produced by very gradual improvements during many years. It is not a selection of varieties, nor of offspring showing combinations of elements from different varieties as a result of crossing, but is simply a selection of individuals. The process is slower and the changes effected are not so great at any one time, but in the end important results may be reached.

Selection of this kind is, of course, the most common, and occurs constantly in nature, especially in connection with the qualities of rust resistance, hardiness against cold, etc. Farmers pretty generally practice a sort of selection of seed corn, and often too of potatoes, for seed. Comparatively little attention, however, is paid to the selection of wheat for seed, although the wheat plant is very susceptible to its environment, furnishing therefore many variations as a basis for excellent results in this line.

It is through this kind of work, but carried on thoroughly and systematically, that Prof. W. M. Hays, of the Minnesota Experiment Station, has attained some very interesting and practical results with the Fife and Velvet Blue Stem varieties of that region. He has practiced rigid selection with these varieties for a number of years, giving special attention to yield and quality of grain as shown by the baker's test. Certain new strains capable of giving to the farmer substantial gains over others have already been produced in this way. He has also developed a method of keeping records which is worthy of the attention of other experimenters.

In the preceding pages the special needs of different wheat districts have been discussed, and also the groups of wheats from which, in crossbreeding, the qualities for satisfying these needs may be secured. One must not forget, however, how much such qualities may be increased in the varieties already grown in the district, and should remember too, that even after great improvements have been secured through hybridization, very careful selection must be practiced in order to maintain the standard of excellence reached, especially if the variety is to be grown under conditions adverse to the production of the particular quality acquired.

Some of the most important qualities of the wheat plant that may readily be increased on any farm simply by selecting seed from those plants which exhibit these qualities to the greatest degree, are yield, drought resistance, winter hardiness, rust resistance, earliness in ripening, quality of the grain in any respect, and nonshattering. If in passing through a field certain plants are noticed which are almost or quite free from rust, while the others are considerably rusted, and the locality should happen to be one in which rust is usually very bad, such heads should by all means be selected, sown separately, and from the progeny the most resistant individuals again selected. It must of course be noted that *all selections for seed should be made in the field.* Even selections for greater yield or for size or quality of grain can not be properly made from the harvested grain. It is fortunate that often two or more qualities may be improved by selecting the same individuals. For example, individuals that are very winter hardy are also likely to be rust resistant in many instances. Great yielding power and nonshattering may also occur in the same individual, while gluten content and drought resistance may exist together in certain others.

In an article by the writer on "Improvements in wheat culture"[1] a simple method is suggested which, if practiced, would enable any farmer to constantly and effectively improve the yield and quality of grain with little trouble, but with great profit in the end. As this method may be employed equally well for the improvement of any other quality of the plant, there is probably no more fitting way of closing the discussion of this topic than to reproduce here the description of that method with such modifications as are necessary to make it applicable for any improvement desired. It is as follows:

Begin practicing the constant use of a wheat-breeding plat of 1 acre or more from which to select seed each year. Locate this plat at different parts of the farm every two or three years, preferably in alternation with clover or other leguminous crops, and give it the best of care. Just before harvest go through a field of a good, hardy, standard variety that has given the best results in the locality, and mark plants that exhibit to the highest degree the special quality which it is desired to increase, such as freedom from rust, fertility of head, or otherwise, and which are at the same time at least as good as the average in other respects. At harvest time cut with a sickle enough of these marked plants for sowing the plat and, after thrashing them, select the largest and most vigorous seed for this purpose, by means of a screen or even by hand picking. Sow the plat early, drilling it at the average rate of about 1¼ bushels per acre. Next season use none of the field crop for seed, but select in the same manner enough of the best plants from this breeding plat for reseeding the plat and use all the remainder for sowing the general crop. In the following season and each succeeding season practice exactly the same method. In this way seed is never taken from the general crop, which can not be given the same care as the small plat, and there is a constant selection of seed which is more and more rigid every year. Moreover, there is no extra labor involved except the small amount required for seed selection each year. Of course the breeding plat should be kept constantly free from rye or other foreign heads and weeds.

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[1] Yearbook United States Department of Agriculture, 1896, pages 489–498; also reprinted.

IMPROVEMENT BY HYBRIDIZATION.

In many instances qualities that are very desirable or even necessary for a particular district are entirely lacking, or at least not present in any appreciable degree, in varieties which are in all other respects admirably adapted to the district. In such instances the improvement of the variety must be accomplished by breeding into it the desired quality from some other sort possessing it to a high degree. Though not so simple a process as that just described, and fraught with much more uncertainty in its operations, hybridization is often absolutely necessary for producing radical changes of great moment, or, in cases of emergency, for satisfying an imperative need, when the ordinary process of selection alone would either be too slow or fail entirely. The possibilities for improvement through hybridization, accompanied by discriminating selection, in the hands of skillful breeders, seem to be practically unlimited, especially in the case of a plant so closely self fertilized as wheat. Nevertheless, comparatively little work of this kind has yet been done with the cereals, and particularly so in this country. Also the greater part of what has been accomplished, though productive of important results, has been of rather an elementary nature.

It may be advisable before continuing the discussion to give first a brief account of some of the principal wheat hybrids produced in this country. Nearly all of these new sorts have proved to be of more or less value in wheat improvement, while a few of them have become well-known factors in developing the industry. The pioneer in the production of wheat hybrids in this country is C. G. Pringle of Charlotte, Vt. Some of the most important of his hybrids are Pringle's No. 4, No. 5, and No. 6, Pringle's Best, and Pringle's Defiance. The last-named variety was produced in Vermont in 1877. In 1878 it was introduced into southern California, and has ever since been a standard sort there, particularly on account of its rust resistance. In the field experiments conducted by this Department this variety and Pringle's No. 5 have always proved to be rather hardy, rust resistant, and productive.

Prof. A. E. Blount, while connected with the Colorado Agricultural College, did much work in crossing wheats, and among a comparatively large number of hybrids produced some that are now not only well known in this country, but are among the most valuable sorts in Australia. They are used by Australian wheat breeders probably more often than any other foreign sorts as the parents of hybrids produced in that country. The most important of Blount's wheats are perhaps the following: Amethyst, Improved Fife, Hornblende, Gypsum, Blount's No. 10, Felspar, Ruby, and Granite. Gypsum (Blount's Lambrigg), Hornblende, Quartz, and Improved Fife are the most popular in Australia. In New Mexico, where field tests of all his hybrids were last made, Ruby and Felspar are now most extensively grown. Blount's No. 10 is much prized in the northern portion of the Pacific coast district, where the variety Oregon No. 10 is probably identical with it. An important characteristic of several of Blount's hybrids is that they are rather rust resistant and it is partly for this reason that they are so much used in Australia. Improved Fife, however, has also an excellent quality of grain.

One of the very best varieties of this country, standing probably next to Fultz in popularity, is Fulcaster. It was produced in 1886 by S. M. Schindel, of Hagerstown, Md., and is a hybrid between Fultz and Lancaster. This variety is a bearded, semihard, red-grained wheat, considerably resistant to leaf rust and drought. It is grown pretty generally throught the country, but especially in the region from Pennsylvania to Oklahoma, including Tennessee and North Carolina to the southward.

Recently Professor Saunders, of Canada, has produced a number of new sorts adapted for growing in the Northern States and Canada. Perhaps a half dozen of these—such as Preston, Percy, Dawn, Alpha, Progress, and Countess—are now pretty well known.

All the hybrids just described have been produced, as a rule, in the most simple way; that is, they were the direct result usually of crosses between varieties comparatively closely allied. That they have met with so much success, therefore, is convincing evidence that most remarkable results must follow extensive hybridization experiments with this cereal when composite methods are employed with parents selected from widely different varieties. No experiments completely of this nature have been made in this country.

Composite crossing, however, is practiced by A. N. Jones, of Newark, N. Y., but always with parents comparatively closely allied. He has without doubt done the most important work in wheat hybridization in this country. Of all American wheat hybrids recently produced, Jones's varieties are to-day most widely used. In composite crossing, after one or more regular simple crosses have been made, one hybrid is either crossed with a fixed variety or with another hybrid, and the offspring of this last cross may be again crossed with another fixed variety or hybrid, and so on. In this way the variations that are always induced even in ordinary simple crosses are of course multiplied many fold, giving practically an unlimited chance of selecting from sporting progeny. The results obtained from composite crossing, therefore, even with varieties closely allied, are not to be compared with those from simple crosses.

Aside from the practice of composite methods, another feature which characterizes Jones's work is the tendency he has shown to adhere to a particular aim in all his operations. The wheats grown in New York and other Eastern States are inclined, on account of the nature of the soil and climate, to be soft and starchy. Recognizing that the best bread flour is made from varieties containing a large proportion of gluten, Jones has given much attention to raising the standard of Eastern varieties in this regard, and has in a large measure succeeded. Of his first varieties the two most popular are his Winter Fife and Early Red Clawson. The former is descended from Fultz, Mediterranean, and Russian Velvet, and is a bald, velvet chaff wheat with amber grains, soft or semihard. It is grown chiefly in the Eastern and North Central States, and would be of great value in the Palouse country were it not for its shattering. Early Red Clawson is a hybrid of Clawson and Golden Cross, the last named being a hybrid of Mediterranean and Clawson. Though in some respects similar to Clawson, it matures earlier and has a stiffer straw. It has a reddish grain. It is a bald, red-chaffed sort, with rather club-shaped, squarely formed heads. In the last eight or ten years it has become very well known in the northern winter-wheat States. Probably the next best known variety is Early Genesee Giant, which has been much grown throughout New York and Pennsylvania. As a good illustration of Jones's method of composite crossing, the full pedigree (fig. 1) of this hybrid, so far as known to the writer, is here given.

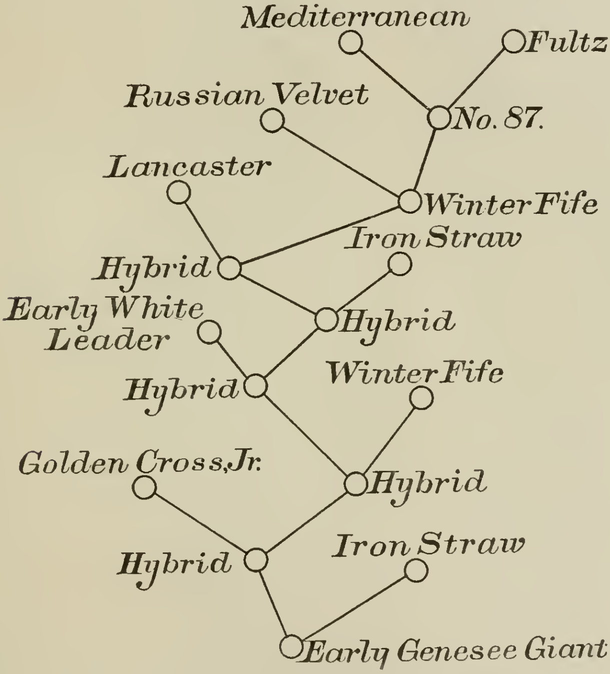

Mediterranean
Fultz
Russian Velvet
No. 87.
Lancaster
Winter Fife
Iron Straw
Hybrid
Early White Leader
Hybrid
Hybrid
Winter Fife
Golden Cross, Jr.
Hybrid
Hybrid
Iron Straw
Early Genesee Giant

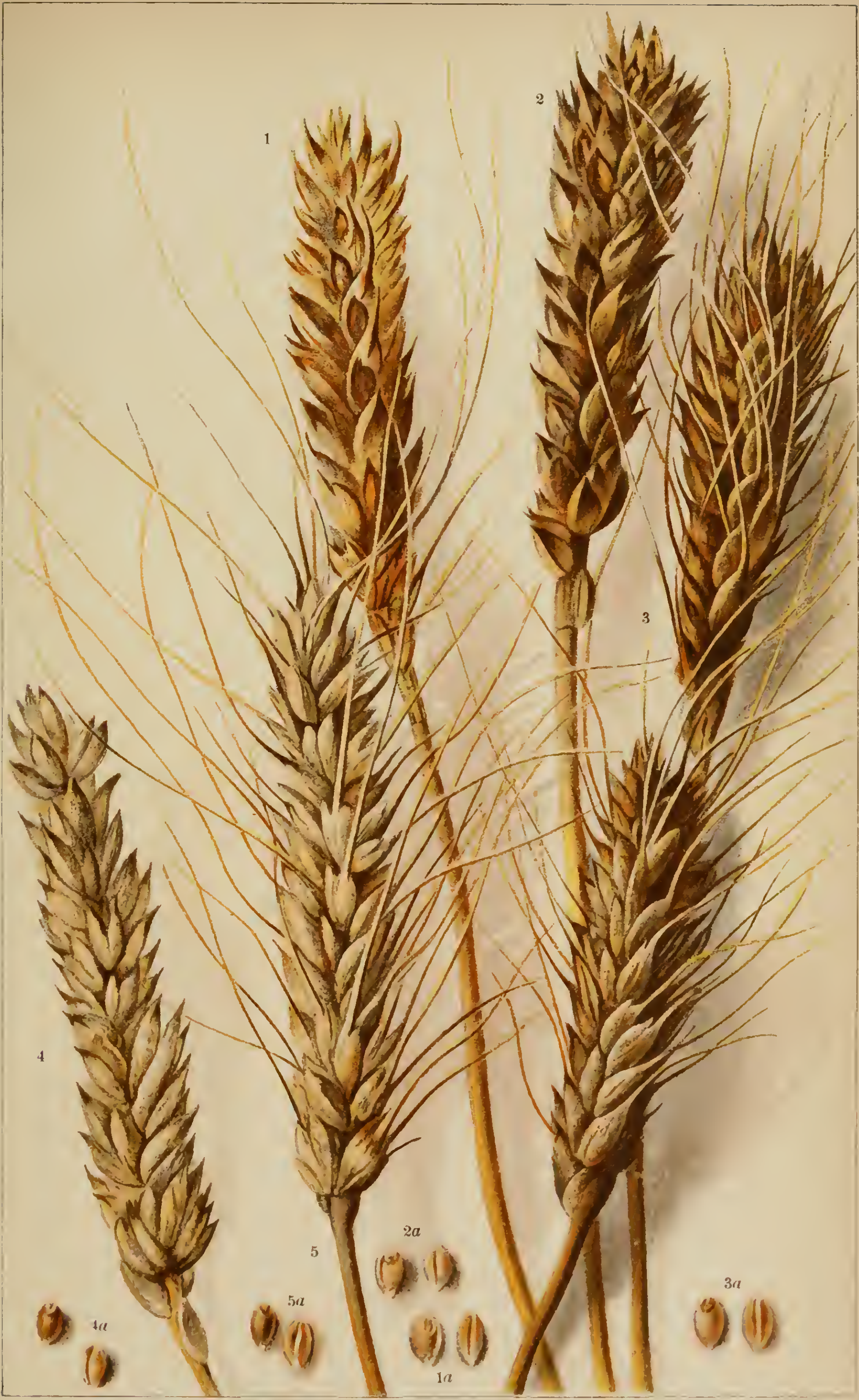
FIG. 1.—Diagram showing pedigree of Early Genesee Giant.

It will be noted that all its ancestors are varieties belonging to the common bread-wheat group. Yet samples of this hybrid show strikingly in various ways the effects of composite crossing, especially exhibiting great improvement in vigor.

In the production of Diamond Grit (Plate IX) and Bearded Winter Fife, Jones has most nearly approached the wheats of the Plains States in gluten content. The former is a direct cross of Jones's Winter Fife with Early Genesee Giant, and is a bearded, white-chaffed, semihard, red-grained variety. Bearded Winter Fife is descended from the Winter Fife as one parent, but is hardier and possesses a grain of better quality. Another hybrid which shows well the advantages of a good ancestry is Early Arcadian (Plate IX). It is a bald, red-chaffed variety, with club and square-shaped heads and light amber grain, and is a direct cross of Early Genesee Giant with Early Red Clawson. It is very productive and of even growth in the field.

But even the method of composite crossing, productive as it is of valuable results, if practiced only with varieties closely allied, as just described, leaves still lacking some important sources for obtaining more rapidly and surely the improvements desired. For anything like perfect attainment of certain qualities it is necessary to practice composite crossing with *varieties of entirely different wheat groups*, a practice which, so far as known to the writer, has only been carried out to any great extent by John Garton, of Newton-le-Willows, England, and William Farrer, of New South Wales. In all the experiments in this country at most but two wheat groups have been drawn from, the common and club wheat groups. But by combining the composite method with the selection of varieties from widely different groups not only are the number of variations induced again multiplied many fold over those induced by the composite method in the same group, but the degree of variation also is much increased. Certain qualities may be obtained in this way that would otherwise even probably not be secured at all. For example, to secure the quality of nonshattering completely it will probably be imperative to introduce it from the spelt or emmer group, while satisfactory resistance to leaf rust must be obtained by crossing with the durums. Besides the direct advantages of increased and multiplied variations induced through selection of parents from different groups for any particular district one is thereby able also to produce sorts adapted for other very different districts, thus allowing his work to be of much wider usefulness. Thus after the production of Jones's Winter Fife, which has been so popular in the Eastern and North Central States, the introduction of the spelt element, without loss of other qualities, might have made it of even greater value for the Palouse country, where it is very much desired, but can not be used because of its shattering.

The wheat plant being so closely self-fertile, there is within it, lying dormant, a wonderful power to vary (a power far greater than in plants cross-fertilized in nature), which is thrown into action when different



D. G. PASSMORE.

A. Hoen & Co. lith. Baltimore

HYBRID WHEATS, EARLY ARCADIAN AND DIAMOND GRIT, BY SIDE OF PARENT VARIETIES.

1, Early Red Clawson (1*a*, grains); 2, Early Arcadian (2*a*, grains); 3, Early Genesee Giant (3*a*, grains); 4, Jones's Winter Fife (4*a*, grains); 5, Diamond Grit (5*a*, grains.)

varieties are artifically crossed. But the enormous amount of variation induced by composite crossing between different wheat groups, though it must be apparent to anyone, can only be appreciated by seeing the results in the field. The writer had the opportunity of observing such results in the experimental plats of the Garton Brothers, in Lincolnshire, England. Their experiments in this line are by far the best illustration of this kind of work in the world. In certain plats were shown the offspring of the second generation from the last cross in cases of series of crosses in which parents were taken from four or even five different wheat groups. In these plats of the second year the progeny had reached the highest degree of variation, and the number of very different forms shown, which came directly, of course, from two parents, were astonishing. There were forms, apparently, of true durums, poulards, spelts, Polish, clubs, and intergradations between these groups, and in many cases characters of every group were easily observable in the same plant. There were large, small, short, long, bearded, and bald heads; velvet and smooth leaves; broad leaves, narrow leaves; leaves glaucous and not glaucous; and plants rusted and not rusted, and of all heights. (Plate X.)

Some of the practical results attained by the Gartons, which are of the greatest economic importance and which serve to show the superiority of their method of operations, should be mentioned. First, it was desired to combine with the yielding capacity of a local variety, rust resistance and tenacity of chaff. By intercrossing this variety with a spelt and a durum these requirements were readily obtained, as witnessed by the writer. But, in addition, the added fertility of the head drawn from the spelt, together, possibly, with the increased vigor of the seed which is often the result of hybridization, still further increased the yield of the original variety. These qualities could not possibly all have been secured by crossing common varieties only, since no varieties of the common group are known to be satisfactorily rust resistant, and only the spelts, emmers, and einkorns are perfectly tenacious of their chaff. In other hybrids great improvement has been made in the hardiness and gluten content of grain, size and fertility of the head, etc., while in nearly all cases the yield has been increased.

Some examples of the results in crossing oats and barley are also very interesting. Common oats have been changed into hulless sorts, but retaining something near the original size of grain, and at the same time one effect of the operations has been to so increase the length of the spikelets as to double the usual yield. The wild oat (*Avena fatua*) has been used successfully in many of these experiments, giving extra vigor and fertility to the new hybrids. In the case of barleys the yield of the six-rowed sorts has been combined with the excellent quality of grain of the two-rowed Chevalier. This combination has been accomplished mainly by forcing fertility in the rows of sterile spikelets of the two-rowed variety. Besides the experiments with cereals, the Gartons have reached many interesting results also with the grasses, beans, and clovers.

The pedigrees of two of the Gartons' hybrid wheats are here given, both in the form of an equation and genealogically for illustration of their method, as follows:

(1) Hybrid = {[Talavera × (Hunter's White × Essex Red)] × [Hungarian Red × (Pedigree White × Black Spelt)]} × [(Pedigree White × Black Spelt) × (Hunter's White × Essex Red)]. (See fig. 2.)

(2) Hybrid = [(Black Spelt × Hardcastle White) × (Mainstay × Hungarian White)] × {[Pedigree Red × (Black Spelt × Hardcastle White)] × (White Chiddam × Hungarian Red)}. (See fig. 3.)

For many years William Farrer has been busily engaged in the work of improving wheats for Australia, especially with respect to rust resistance, and has not only practiced composite crossing, but has found it necessary to use in many cases parent forms from different wheat groups, including common wheats, club wheats, durums, and poulards. His new varieties, although chiefly adapted to Australian conditions, are many of them most excellent ones, which show their high breeding to a marked degree, and represent an enormous amount of work. Among the very many parent varieties used in his work are the following excellent sorts: Improved Fife, Gypsum, Tourmaline, Hornblende, Quartz, Early Japanese, Beloturka, Medeah, Sicilian Red Square-head, D'Arblay's Hungarian, Zimmerman, Ward's Prolific, Fultz, Ward's White, Blount's Fife, several early maturing Indian varieties, and others that might be considered just as good as these. The following pedigree of one of his hybrids will illustrate his methods:

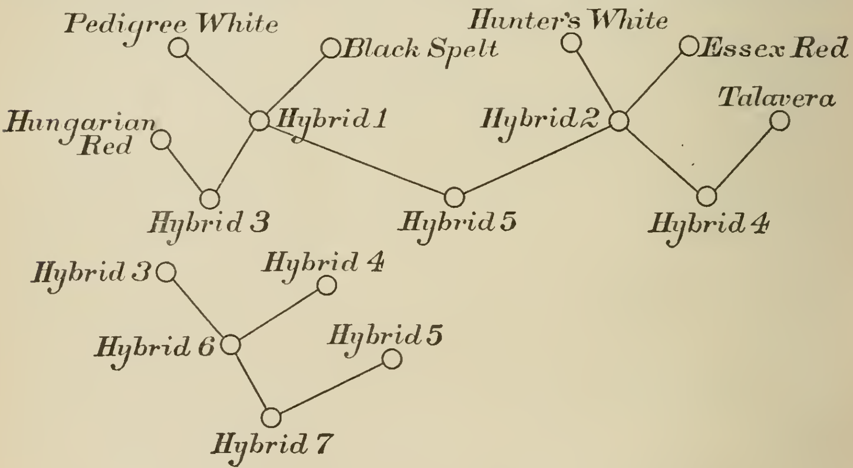
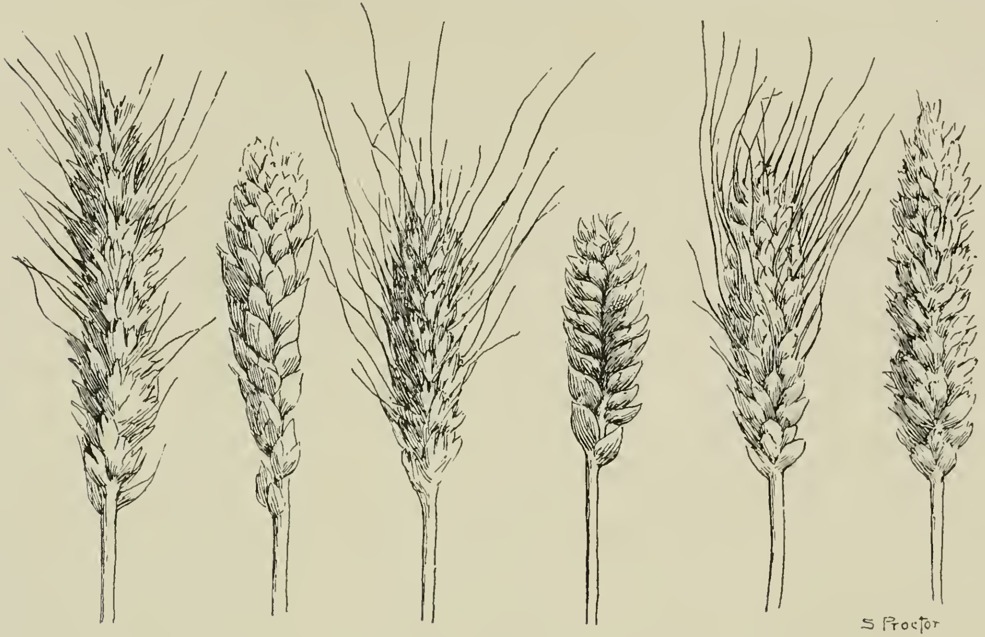


FIG. 2.—Diagram showing pedigree of one of the Gartons' hybrid wheats.



A COMPOSITE CROSS BY THE GARTONS, SHOWING SAMPLES OF THE PROGENY OF THE LAST CROSS.

(Redrawn from Trans. Highl. and Agric. Soc. of Scotland, Series V, Vol. VI, Pl. V.)

Hybrid = { [(Medeah × Gypsum) × Hornblende] × [Hornblende × Ward's White] } × Improved Fife. (See fig. 4.)

Medeah is a North African durum wheat. The others are common bread wheats. This new hybrid has been tested by the writer in the field experiments of this department, and was found to be a vigorous sort.

Among Continental breeders probably the most important work with cereals has been done by W. Rimpau, of Schlanstedt, Germany. Though not characterized by the use of composite methods, Rimpau's work shows a number of important examples of the results obtained by crossing with parents from different wheat groups. Some of the most interesting of the crosses showing various forms similar to the parents and intergrading as to form, color, etc., are the following: Rivett's Bearded Spelt (poulard) × Red German Bearded, Rivett's Bearded × Square-head (club group), and Mainstay × Square-head.[1]

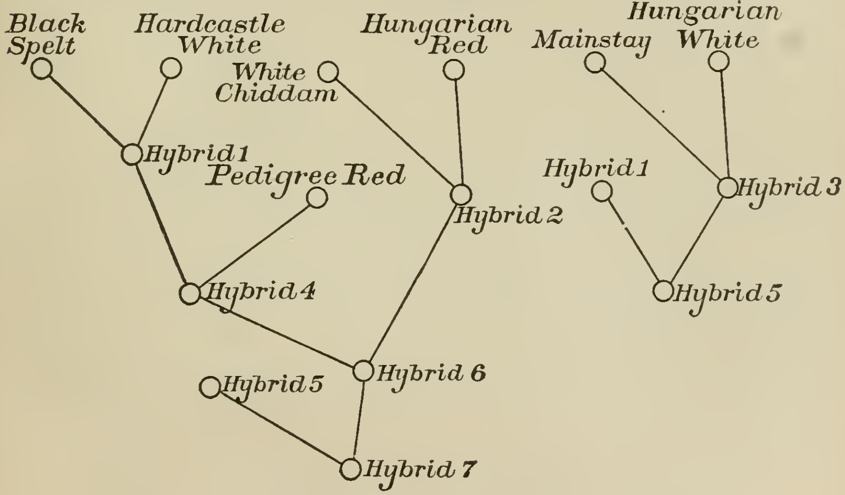

Black Spelt
Hardcastle White
White Chiddam
Hungarian Red
Mainstay
Hungarian White
Hybrid 1
Pedigree Red
Hybrid 2
Hybrid 1
Hybrid 3
Hybrid 4
Hybrid 5
Hybrid 6
Hybrid 5
Hybrid 7

FIG. 3.—Diagram showing pedigree of one of the Gartons' hybrid wheats.

As already shown in the earlier part of this bulletin, wheat is, of all the principal cultivated crops, probably the most influenced by its environment. Connect with this the fact also of its close self-fertilization, and it is readily explained why there are so many different varieties, each best adapted to its particular district. The same variety taken to localities characterized by widely different conditions will gradually change to suit the new conditions, thus giving origin to different strains. At the same time new hybrids, when well fixed, are not likely to be broken up by subsequent natural crosses, as in the case of some other species. It is important, therefore, that all hybrids intended for a particular district should either be produced in that district or transferred there before they have become fixed, in order that the careful selection necessary may be continued in accordance with the tendencies developed under the influence of the new conditions.

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[1] For an interesting account of some of Rimpau's work, written by himself, see "Kreuzungsprodukte landwirthschaftlicher Kulturpflanzen." Landwirthschaftliche Jahrbücher, Bd. XX, S. 335–371 (Illus.), 1891.

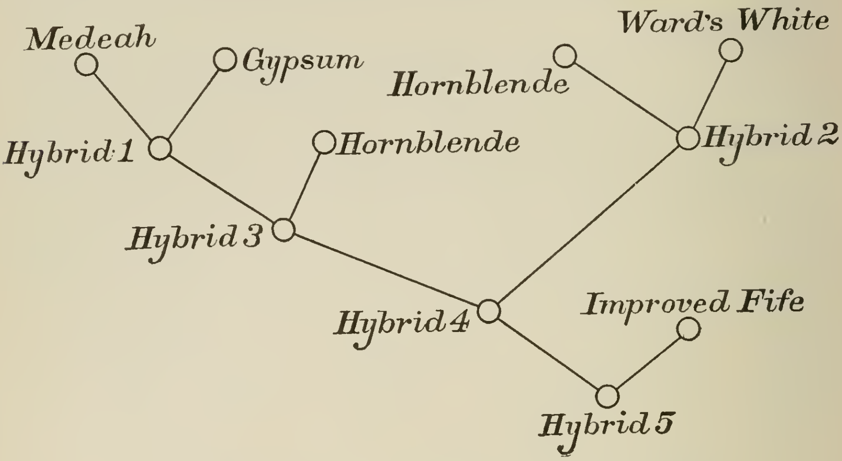
Medeah
Gypsum
Hornblende
Ward's White
Hybrid 1
Hornblende
Hybrid 2
Hybrid 3
Hybrid 4
Improved Fife
Hybrid 5

Fig. 4.—Diagram showing pedigree of one of Farrer's hybrid wheats.

Another matter of importance should be noted before leaving this topic. It was supposed for a time, and is still supposed by some, that varieties from different wheat groups will not cross with each other. Often this is true if it is attempted to cross them directly; but it shows another great advantage of composite crossing that if these same varieties are first crossed with others of the same group, or with those of groups more nearly allied, the resulting progeny will cross more readily with that of a widely different group. For example, instead of attempting to cross a common wheat with a spelt, the desired result would be more certainly and easily attained by means of a composite cross similar to that shown in the accompanying diagram (fig. 5), and at the same time there is a much better chance offered for selection because of the increased amount of variation thereby induced.

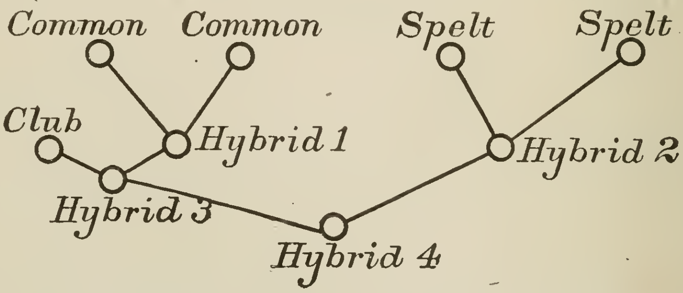
Common
Common
Spelt
Spelt
Club
Hybrid 1
Hybrid 2
Hybrid 3
Hybrid 4

Fig. 5.—Diagram showing hypothetical cross of wheat and spelt.

Through long, natural "in-and-in breeding" the qualities of the variety have become specialized, as it were, in harmony with the conditions of the environment, and do not readily amalgamate with those of a widely different sort. But once produce variation among these qualities by means of crosses with allied sorts, and it becomes easier to blend them with those of very different sorts.

SUMMARY.

1. As a foundation for rational wheat improvement, a knowledge is required of (1) the characteristics and needs of different wheat districts, and (2) the characteristic qualities of the natural groups of wheats.

2. On the basis of conditions of soil and climate and the nature of the varieties adapted to these conditions, the United States may be considered to be divided into eight wheat districts as follows: (1) Soft Wheat district, including mainly the Middle and New England States; (2) Semihard Winter Wheat district, including Ohio, Indiana, Illinois, Michigan, and a small part of Wisconsin; (3) Southern Wheat district, including approximately the Southern States; (4) Hard Spring Wheat district, covering the northern portion of the States of the Plains; (5) Hard Winter Wheat district, covering the central portion of the States of the Plains; (6) Durum Wheat district, covering the southern portion of the States of the Plains; (7) Irrigated Wheat district, including approximately the Rocky Mountain and Basin States, and (8) White Wheat district, including the Pacific Coast States.

3. Certain general needs, such as early maturity and greater yielding power, are common to all these districts and must be kept constantly in mind in connection with all efforts made to improve varieties. Other characteristics and needs are more special and are stated herewith under headings of the different districts.

4. Soft Wheat district:

(*a*) Present average yield per acre, about 14⅔ bushels.
(*b*) Chief varieties now grown:
    Fultz,
    Fulcaster,
    Early Genesee Giant,
    Mediterranean,
    Early Red Clawson,
    Longberry,
    Jones's Winter Fife,
    Red Wonder,
    Gold Coin,
    Blue Stem.
(*c*) Needs of the grower:
    Harder-grained, more glutinous varieties.
    Hardier winter varieties for the most northern portions.
    Early maturity.
    Rust resistance.

5. Semihard Winter Wheat district:

(*a*) Present average yield per acre, about 14 bushels.
(*b*) Chief varieties now grown:
    Fultz,
    Poole,
    Rudy,
    Valley,
    Nigger,
    Dawson's Golden Chaff,
    Early Red Clawson.

(*c*) Needs of the grower:
Hardness of grain.
Rust resistance.
Hardy winter varieties.

6. Southern Wheat district:

(*a*) Present average yield per acre, about $9\frac{2}{3}$ bushels.
(*b*) Chief varieties at present grown:
Fultz,
Fulcaster,
Red May,
Rice,
Everett's High Grade,
Boughton,
Currell's Prolific,
Purple Straw.
(*c*) Needs of the grower:
Rust resistance.
Early maturity.
Resistance to late spring frosts.
Stiffness of straw.

7. Hard Spring Wheat district:

(*a*) Present average yield per acre, about 13 bushels.
(*b*) Chief varieties at present grown:
Saskatchewan Fife,
Scotch Fife,
Powers Fife,
Wellman's Fife,
Hayne's Blue Stem,
Bolton's Blue Stem.
(*c*) Needs of the grower:
Early maturity.
Rust resistance.
Drought resistance.
Hardy winter varieties.

8. Hard Winter Wheat district:

(*a*) Present average yield per acre, about $12\frac{2}{3}$ bushels.
(*b*) Chief varieties at present grown:
Turkey,
Fulcaster,
May,
Zimmerman,
Fultz.
(*c*) Needs of the grower:
Drought resistance.
Hardy winter varieties.
Early maturity.

9. Durum Wheat district:

(*a*) Present average yield per acre, $11\frac{1}{2}$ bushels.
(*b*) Chief varieties at present grown:
Mediterranean,
Nicaragua,
Fulcaster,
Turkey.
(*c*) Needs of the grower:
Macaroni varieties.
Drought resistance.
Rust resistance.
Early maturity.

10. Irrigated Wheat district:

(*a*) Present average yield per acre about 21 bushels.
(*b*) Chief varieties at present grown:
    Sonora,
    Taos,
    Felspar,
    Little Club,
    Defiance,
    Amethyst.
(*c*) Needs of the grower:
    Increase of the gluten content.
    Early maturity.

11. White Wheat district:

(*a*) Present average yield per acre about 14⅞ bushels.
(*b*) Chief varieties at present grown:
    Australian,
    California Club,
    Sonora,
    Oregon Red Chaff,
    Foise,
    Palouse Blue Stem,
    Palouse Red Chaff,
    White Winter,
    Little Club.
(*c*) Needs of the grower:
    Early maturity.
    Nonshattering varieties.
    Hardy winter varieties in the colder portions.

12. The cultivated varieties of wheat are naturally divided into eight rather distinct groups, corresponding to eight botanic species, as follows: (1) Common Bread Wheat (*Triticum vulgare*), (2) Club or Square-head (*T. compactum*), (3) Poulard (*T. turgidum*), (4) Durum (*T. durum*), (5) Polish Wheat (*T. polonicum*), (6) Spelt (*T. spelta*), (7) Emmer (*T. dicoccum*), and (8) Einkorn (*T. monococcum*). The special characteristics of these groups of wheats that are of prime importance in the work of wheat breeding are here given:

(1) Common Bread Wheat group:
    (*a*) Excellence of gluten content for bread making.
    (*b*) Excellence of certain varieties for cracker making.
    (*c*) Yielding power of certain sorts.
    (*d*) Rust resistance (in some varieties).
    (*e*) Winter hardiness of certain varieties.
    (*f*) Resistance to drought of certain varieties.
    (*g*) Early maturity (in some varieties).
(2) Club or Square-head group:
    (*a*) Great yielding power.
    (*b*) Stiffness of straw.
    (*c*) Freedom from shattering.
    (*d*) Early maturity (in some varieties).
    (*e*) Drought resistance (in some varieties).
    (*f*) Excellence of certain sorts for making crackers and breakfast foods.
(3) Poulard group:
    (*a*) Excellence of certain varieties for making macaroni.
    (*b*) Resistance to orange leaf rust.
    (*c*) Resistance to drought.
    (*d*) Stiffness of straw.

(4) Durum group:
  - (*a*) Excellence of gluten content for making macaroni and other pastes.
  - (*b*) Resistance to drought.
  - (*c*) Resistance to orange leaf rust.

(5) Polish Wheat group:
  - (*a*) Quality of gluten content for making macaroni.
  - (*b*) Resistance to drought.
  - (*c*) Resistance to orange leaf rust.

(6) Spelt group:
  - Desirable qualities—
    - (*a*) Ability to hold the grain in the head.
    - (*b*) Constancy in fertility.
    - (*c*) Hardiness of certain winter sorts.
  - Undesirable qualities—
    - (*d*) Brittleness of head.
    - (*e*) Rust liability.

(7) Emmer group:
  - Desirable qualities—
    - (*a*) Ability to hold the grain in the head.
    - (*b*) Drought resistance.
    - (*c*) Resistance to orange leaf rust.
  - Undesirable qualities—
    - (*d*) Brittleness of the head.
    - (*e*) Adaptability only for spring sowing, as a rule.

(8) Einkorn group:
  - Desirable qualities—
    - (*a*) Ability to hold the grain in the head.
    - (*b*) Resistance to orange leaf rust.
    - (*c*) Hardiness.
    - (*d*) Resistance to drought.
    - (*e*) Stiffness of straw.
  - Undesirable quality—
    - (*f*) Brittleness of the head.

13. Wheats may also be grouped geographically. On this basis groups of varieties having certain special qualities are located approximately as follows:

(*a*) Starchy white wheats: Pacific Coast and Rocky Mountain States, Chile, Turkestan, Australia, and India.

(*b*) Amber or reddish grained wheats, also starchy: Eastern States, western and northern Europe, India, Japan, and Australia.

(*c*) Excellence of gluten content for making the best bread: Northern and Central States of the Plains, Canada, eastern and southern Russia, Hungary, Roumania, and southern Argentina.

(*d*) Resistance to orange leaf rust: Southern Russia, Mediterranean and Black Sea regions, and Australia.

(*e*) Excellence of gluten content for making macaroni: Southern Russia, Algeria, and the Mediterranean region in general.

(*f*) Stiffness of straw preventing lodging: Pacific Coast States, Japan, Turkestan, Mediterranean region, and Australia.

(*g*) Yielding power (at least in proportion to size of head): Pacific Coast States, Chile, and Turkestan.

(*h*) Nonshattering varieties: Pacific Coast States, Chile, Turkestan, Germany (spelts), and East Russia (emmers.)

(*i*) Constancy in fertility: Germany (spelts) and southern Europe.
(*j*) Early maturity: Japan, Australia, and India.
(*k*) Resistance to drought and heat: East and South Russia, Kirghiz Steppes, Turkestan, and southern Mediterranean region.
(*l*) Resistance to drought and cold: East Russia.

14. Of the many wheat introductions made into this country in the past, the following are among those of the greatest moment, and which have completely revolutionized the wheat industry in places:

(*a*) Mediterranean, introduced first in 1819.
(*b*) Fife wheats, introduced first into Canada and then into the northern States of the Plains.
(*c*) Turkey wheat, first introduced into Kansas about twenty-five years ago from Taurida, in Russia.
(*d*) The California Club, Australian, and Sonora, introduced into the Pacific coast States.

15. Field experiments of the Department have shown that in the common bread-wheat group there is a very close constant relation between the autumn condition of the young plant on the one hand and winter hardiness and quality of grain on the other.

16. Wheat is very susceptible to changes of environment, but especially in regard to color, hardiness, and chemical composition of the grain.

17. In general, regions possessing black prairie soils and characterized by violent climatic extremes, especially extremes of heat and drought, produce wheats that are hardiest, have the hardest grains, and are the best in quantity and quality of gluten content.

18. Considering all qualities, the best wheats of the world are of Russian origin, coming particularly from eastern and southern Russia, the Kirghiz steppes, and Turkestan. Of Russian varieties so far known, the following are among the best, if not the very best:

Arnautka,
Kubanka,
Ghirka Winter,
Crimean,
Sarui-bug-dai,
Mennonite,
Chernokoloska,
Turkey,
Ghirka Spring,
Russian,
Buivola,
Kubanka Red Winter,
Yx,
Beloturka.

19. The earliest ripening wheats are often dwarfed. The following varieties are among the best of this class:

Yemide,
Onigara,
Shiro-Yemidashi,
Kintama,
Kathia,
Roseworthy,
Early Baart,
Early Japanese,
Japanese No. 2,
Nashi,
Allora Spring,
Steinwedel,
King's Jubilee.

20. The following varieties are among the best known of the durum and poulard groups:

Arnautka,
Kubanka,
Beloturka,
Chernokoloska,
Medeah,
Sarui-bug-dai,
Cretan,
Galland's Hybrid,
El Safra,
Petanielle noire de Nice,
Volo,
Missogen,
Atalanti,
Nicaragua.

21. Common bread wheats can not be depended upon to resist rust, but the best in this regard are probably the following:

Turkey,
Pringle's Defiance,
Rieti,
Pringle's No. 5,
Nashi,
Saskatchewan Fife,
Theiss,
Bellevue Talavera,
Arnold's Hybrid,
Crimean,
Oregon Club,
Odessa,
Mennonite,
Velvet Blue Stem,
Sonora,
Prolifero,
Mediterranean,
Deitz Longberry.

22. Einkorns resist leaf rust completely, and emmers resist it to a high degree.

23. Some of the very hardiest winter varieties so far tried in this country are:

Turkey,
Yx,
Crimean,
Ghirka Winter,
Bearded Winter.

24. Club wheats are usually soft-grained and tender sorts, adapted only to mild climates like that of California. Among the best of this group are:

Little Club,
California Club,
Herisson barbu,
Palouse Red Chaff,
Chili Club,
Sicilian Red Square-head,
Herisson sans barbes.

25. Some of the most popular and valuable wheats of our country have been produced by simple selection, though in some cases the indications are strong that they were originally the result of natural crossing. The best known of such varieties are:

Fultz,
Clawson,
Gold Coin,
Rudy,
Wellman's Fife,
Currell's Prolific.

26. Selection plays far the most important part in wheat breeding, and necessitates on the part of the experimenter a thorough knowledge of varieties and their relations to each other and to their environment.

27. Simple selection of individuals, however, for the improvement of the same variety can and should be practiced on every farm. Very little extra time or trouble is required, but the gain is great.

28. Among the most valuable wheats of the United States that have been produced through hybridization are the following:

Fulcaster,
Gypsum,
Improved Fife,
Quartz,
Ruby,
Jones's Winter Fife,
Early Genesee Giant,
Early Arcadian,
Pringle's Defiance,
Pringle's No.5,
Hornblende,
Felspar,
Blount's No. 10,
Diamond Grit,
Early Red Clawson,
Early White Leader.

29. For the most complete success in wheat improvement through hybridization it is necessary to practice composite crossing with parents selected from widely different wheat groups.

30. The wheat plant is so closely self-fertilized in nature that the practice of composite crossing produces some most interesting and remarkable results. There is apparently no end to the variations exhibited by the sporting progeny in such cases, and, accompanied by discriminating selection, the possibilities of wheat improvement in this way are practically unlimited.

INDEX OF VARIETIES.

Page.
Algerian 44, 45
Allora Spring 43, 44, 45, 63, 81
Alpha 70
Alsace 44, 45, 61, 63
American 44, 45
American Bronze 44, 45
Ames 44, 45
Amethyst 21, 44, 45, 69, 79
Amidonnier. (*See* Emmer.)
Arnautka 19, 31, 44, 45, 62, 63, 81, 82
Arnold's Hybrid 44, 45, 63, 82
A six rangs 44, 45
Assiniboine Fife 44, 45
Astrakhan 32
Atalanti 44, 45, 63, 82
Australian 22, 25, 40, 43, 44, 45, 79, 81
Australian Indian 44, 45
Australian Purple Straw 44, 45
Baggi 44, 45
Banat 28, 44, 45, 63
Barletta 44, 45
Barley wheats. (*See* Durum wheats.)
Basalt 44, 45
Bauchiger Weizen. (*See* Poulard wheats.)
Bearded Winter 44, 45, 62, 63, 82
Bearded Winter Fife 72
Bellevue Talavera 44, 45, 63, 82
Belokoloska 44, 45
Beloturka 15, 44, 45, 62, 63, 74, 81, 82
Berthoud 44, 45
Bianehetta 44, 45
Big English 44, 45
Big Frame 44, 45
Black Spelt 74, 75
Black Velvet 44, 45
Blé petanielle. (*See* Poulard wheats.)
Blount's Fife 44, 45, 74
Blount's No. 10 44, 45, 69, 70, 83
Blue Stem 13, 16, 44, 45, 77
Bolton's Blue Stem 17, 44, 45, 66, 78
Boughton 15, 78
Bread wheats 7, 26, 28, 30, 31, 32, 33, 37, 43, 63, 71, 74, 75, 76, 79
Buea Nera 44. 45
Buckeye 44, 45
Budapest 39, 46, 47
Buivola 28, 62, 81
California Club 25, 63, 79, 81, 82
Candeal Redondo 46, 47
Canning Downs 14, 46, 47, 63
Cape 46, 47
Cartagena 46, 47
Chernokoloska 46, 47, 62, 63, 81, 82
Chernouska 46, 47
Chiddam de Mars rouge 46, 47
Chili 46, 47
Chili Club 22, 46, 47, 63, 82
China Red 46, 47
China Tea 46, 47
China White 46, 47
Chinese 46, 47
Chubut 46, 47, 63
Clawson 46, 47, 59, 65, 66, 71, 82
Club wheats 22, 23, 24, 28, 29, 37, 40, 43, 63, 73, 74, 75, 76, 79, 82
Common wheats. (*See* Bread wheats.)
Composite wheats 30
Countess 70
Cretan 46, 47, 63, 82
Crimean 28, 46, 47, 62, 63, 81, 82
Currell's Prolific 15, 46, 47, 78, 82

Page.
Dallas 46, 47
D'Arblay's Hungarian 46, 47, 74
Daruma 46, 47, 63
Dattel 46, 47
Dawn 70
Dawson's Golden Chaff 14, 39, 46, 47, 77
Defiance 15, 21, 22, 46, 47, 79
Deitz 46, 47
Deitz Longberry 63, 82
De la Basse 46, 47
Diamond Grit 21, 46, 47, 72, 83
Diehl Mediterranean 46, 47, 66
Dinkel. (*See* Spelt.)
Dividenden 46, 47
Duro di Apulia 46, 47
Durum wheats 8, 15, 19, 26, 29, 30, 31, 32, 33, 37, 40, 43, 60, 63, 72, 73, 74, 75, 79, 80
Early Arcadian 46, 47, 72, 83
Early Baart 46, 47, 63, 81
Early Genesee Giant 13, 43, 46, 47, 71, 72, 77, 83
Early Japanese 48, 49, 63, 74, 81
Early May 43, 48, 49
Early Red Clawson 13, 14, 48, 49, 65, 71, 72, 77, 83
Early Rice 48, 49
Early White Leader 71, 83
Einkorn 35, 36, 37, 43, 48, 49, 63, 73, 79, 80, 82
El Safra 48, 49, 63, 82
Emmer 30, 33, 34, 35, 37, 43, 72, 73, 79, 80, 82
English wheats. (*See* Poulard wheats.)
Engrain. (*See* Einkorn.)
Engrain double 36, 48, 49
Entre Rios 48, 49
Epeautre 33
Essex Red 74
Everett's High Grade 15, 48, 49, 78
Farquhar 48, 49
Farrer's Durum 48, 49
Felspar 21, 48, 49, 69, 70, 79, 83
Fern 48. 49
Fife 16, 21, 28, 31, 38, 65, 67, 81
Flourelle 48, 49
Fluor Spar 48, 49
Foise 23, 25, 79
Frampton 48, 49
Frances 48, 49
Frankenstein 48, 49
Fuleaster 13, 15, 18, 20, 28, 48, 49, 63, 70, 77, 78, 83
Fultz 13, 14, 15, 18, 39, 48, 49, 63, 65, 70, 71, 74, 77, 78, 82
Galland's Hybrid 48, 49, 63, 82
German Amber 48, 49
German Emperor 48, 49
Gerstenweizen. (*See* Durum wheats.)
Gharnovka 48, 49
Ghirka 28, 39
Ghirka Spring 56, 57, 62, 63, 81
Ghirka Winter 58, 62, 63, 81, 82
Giant Rye. (*See* Polish wheats.)
Glasgow 38
Glyndon 673 48, 49
Glyndon 811 48, 49
Gold Coin 13, 16, 48, 49, 66, 77, 82
Golden Cross 48, 49, 71
Golden Cross Jr 71
Goldene Aue 48, 49
Golden Gate Club 22
Graf Walderdorff's Regenerated 48, 49
Granite 69
Grass. (*See* Odessa.)
Gypsum 48, 49, 69, 74, 75, 76, 83
Haffkani 48, 49

Page.
Hallett's Pedigree 48, 49
Hardcastle White 74, 75
Hayne's Blue Stem 17, 48, 49, 66, 67, 78
Herisson barbu 50, 51, 63, 82
Herisson sans barbes 50, 51, 63, 82
Hickling 50, 51
Hopetown 50, 51
Hornblende 50, 51, 69, 74, 75, 76, 83
Hudson's Early Purple Straw 50, 51
Hundred Fold 30
Hungarian Red 74, 75
Hungarian White 74
Hunter's White 74
Igel mit Grannen 50, 51
Igel ohne Grannen 50, 51
Imperial 50, 51
Improved Fife 50, 51, 61, 69, 70, 74, 75, 76, 83
Iron Straw 71
Japanese No. 1 50, 51, 63
Japanese No. 2 50, 51, 63, 81
Japanese No. 4 50, 51, 63
Jejar de Valencia 50, 51
Jerusalem Rye. (*See* Polish wheats.)
Jones's Square-head 50, 51
Jones's Winter Fife 13, 50, 51, 71, 72, 77, 83
Kastamuni 50, 51
Kathia 50, 51, 63, 81
Khel 50, 51
King's Jubilee 43, 50, 51, 63, 81
Kinney 50, 51
Kintama 50, 51, 63, 81
Krasnokoloska 50, 51
Kubanka 19, 31, 50, 51, 61, 62, 63, 81, 82
Kubanka Red Winter 62, 81
Kubb 50, 51
Ladoga 50, 51
Lal 50, 51
Lamed 50, 51
Lancaster 50, 51, 63, 65, 70, 71
Lehigh 50, 51
Linaza 50, 51
Little Club 21, 24, 25, 43, 50, 51, 60, 63, 79, 82
Longberry 13, 77
Lost Nation 50, 51
Macaroni wheat. (*See* Durum, Poulard, and Polish wheats.)
Mainstay 74, 75
May 15, 18, 78
McKissick's Fife 50, 51
Mealy 52, 53
Medeah 52, 53, 63, 74, 75, 76, 82
Mediterranean 13, 19, 20, 28, 38, 52, 53, 63, 71, 77, 78, 81, 82
Meekins 52, 53
Melka 52, 53
Mennonite 52, 53, 62, 63, 81, 82
Minnesota Fife 52, 53
Miracle 30
Mirado 52, 53
Missogen 52, 53, 63, 82
Moscow 52, 53
Mundia 52, 53
Mureia 52, 53
Muzaffarnagar 52, 53
Nab-el-bel 52, 53
Nashi 52, 53, 63, 81, 82
Nicaragua 15, 19, 20, 31, 32, 40, 63, 78, 82
Nigger 14, 52, 53, 77
Noé 52, 53
Nonette de Lausanne 52, 53
Nonpareil 52, 53
No. 6. (*See* Gold Coin.)
Odessa 39, 52, 53, 62, 63, 82
Onigara 52, 53, 63, 81
Oregon Club 52, 53, 63, 82
Oregon No. 10 70
Oregon Red Chaff 23, 25, 79
Palouse Blue Stem 16, 22, 24, 25, 40, 42, 52, 53, 60, 61, 79
Palouse Red Chaff 22, 25, 63, 79, 82
Pedigree Red 74, 75
Pedigree White 74
Penquite's Velvet Chaff 52, 53
Percy 70
Petanielle noire de Nice 52, 53, 63, 82
Pilli 52, 53
Pissi Hydrabadi 52, 53

Page.
Polba. (*See* Emmer.)
Polish wheat 31, 32, 33, 37, 43, 52, 53, 63, 73, 79, 80
Poole 14, 39, 52, 53, 59, 77
Poulard wheats 8, 26, 29, 30, 31, 37, 43, 60, 63, 73, 74, 75, 79
Power's Fife 17, 52, 53, 66, 78
Preston 70
Pringle's Best 69
Pringle's Defiance 52, 53, 63, 69, 82, 83
Pringle's No. 4 69
Pringle's No. 5 52, 53, 63, 69, 82, 83
Pringle's No. 6 69
Probsteier 52, 53
Progress 70
Prolifero 52, 53, 63, 82
Prophet 52, 53
Propo 22, 54, 55
Pulavka 54, 55
Purple Straw 15, 40, 54, 55, 78
Quartz 54, 55, 69, 74, 83
Rattling Jack 54, 55
Red Bearded 54, 55
Red Chaff. (*See* Oregon Red Chaff and Palouse Red Chaff.)
Red Chaff Club. (*See* Palouse Red Chaff.)
Reddish White Bearded 54, 55
Red Fife 54, 55
Red German Bearded 75
Red May 15, 78
Red Provence 54, 55
Red Spring 54, 55
Red Tyrol 54, 55
Red Winter 54, 55, 62, 63
Red Wonder 13, 77
Rice 15, 54, 55, 78
Rieti 54, 55, 63, 82
Rio Grande 54, 55
Rivett's Bearded Spelt 75
Rivet wheats. (*See* Poulard wheats.)
Roseworthy 54, 55, 63, 81
Ruby 69, 70, 83
Rudy 14, 23, 54, 55, 65, 77, 82
Russian 62, 81
Russian Hard 54, 55
Russian Spring 54, 55
Russian Velvet 71
Rye Wheat 54, 55
Safeed 54, 55
Saida 54, 55
Saldomé 54, 55
Salt Lake Club 22
Samara 54, 55
Sandomir 54, 55, 62
Saratov 54, 55
Sarui-bug-dai 54, 55, 62, 63, 81, 82
Saskatchewan Fife 17, 54, 55, 63, 65, 78, 82
Saumur Winter 54, 55
Scotch 38
Scotch Fife 17, 54, 55, 61, 65, 78
Seneca Chief 54, 55
Seven-headed 30, 54, 55
Shirosawa 54, 55
Shiro-yemidashi 54, 55, 63, 81
Sicilian Red Square-head 54, 55, 63, 74, 82
Sindhi 56, 57
Sonora 21, 22, 25, 40, 56, 57, 63, 79, 81, 82
Soules 56, 57
Spelt 33, 34, 35, 37, 43, 72, 73, 76, 79, 80, 81
Spelz. (*See* Spelt.)
Spring Ghirka (*see also* Ghirka Spring) 56, 57
Square-head 75
Square-head wheats. (*See* Club wheats.)
Steinwedel 56, 57, 63, 81
Swamp 56, 57
Taganrog 56, 57
Talavera 56, 57, 74
Tangarotto 56, 57, 63
Taos 21, 56, 57, 79
Tasmanian Red 56, 57, 63
Theiss 28, 39, 56, 57, 63, 82
Tourmaline 56, 57, 74
Touzelle 56, 57
Trimenia 56, 57
Triticum 25, 26
Triticum compactum 22, 26, 28, 29, 79
Triticum compositum 30
Triticum dicoccum 26, 30, 33, 34, 79

Page.
Triticum durum, 8, 26, 29, 30, 79
Triticum monococcum, 26, 35, 79
Triticum polonicum, 26, 32, 79
Triticum spelta, 26, 33, 79
Triticum turgidum, 8, 26, 29, 79
Triticum vulgare, 7, 8, 26, 29, 79
Turkey, 17, 18, 20, 21, 28, 31, 39, 56, 57, 59, 60, 62, 63, 78, 81, 82
Tuscan, 56, 57
Ulka, 28, 39, 62
Urtoba, 56, 57
Valley, 14, 56, 57, 63, 77
Varesotto, 56, 57
Velvet Blue Stem, 16, 28, 31, 56, 57, 63, 65, 67, 82
Velvet Chaff, 56, 57
Victoria d'Automne, 56, 57
Victorian Defiance, 56, 57
Volo, 56, 57, 63, 82
Vyssoko-Litovsk, 56, 57
Walla Walla, 56, 57

Page.
Walker, 56, 57
Ward's Prolific, 56, 57, 74
Ward's White, 74, 75, 76
Wellman's Fife, 17, 58, 65, 66, 67, 78, 82
White Chiddam, 74, 75
White Clawson. (*See* Clawson.)
White Michigan, 39
White Polish, 32
White Tuscan, 58
White Winter, 25, 58, 79
Wild Goose, 40, 58, 63
Winter Fife. (*See* Jones's Winter Fife.)
Winter Ghirka (*see, also*, Ghirka Winter), 58
Wonder, 30
Wyandotte Red, 58
Yemide, 43, 58, 63, 81
Yx, 58, 62, 63, 81, 82
Zaruta, 58
Zimmerman, 18, 58, 74, 78

BULLETIN No. 25. V. P. P.—78.

U. S. DEPARTMENT OF AGRICULTURE,

DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY.

B. T. GALLOWAY, Chief.

SOME DISEASES OF NEW ENGLAND CONIFERS:

A PRELIMINARY REPORT.

BY

HERMANN VON SCHRENK,
Instructor in Botany, Henry Shaw School of Botany,
Special Agent, Division of Vegetable Physiology and Pathology.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1900.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Division of Vegetable Physiology and Pathology,
Washington, D. C., August 8, 1900.

Sir: I respectfully transmit herewith a paper prepared by Dr. Hermann von Schrenk, special agent of this Division, on Some Diseases of New England Conifers. The investigations described were carried on in cooperation with the Division of Forestry of this Department and the Shaw School of Botany, of St. Louis, Mo., and are of special interest at this time, in view of the increasing demand for information on forest problems. I respectfully recommend that the paper be published as Bulletin No. 25 of this Division.

Dr. von Schrenk desires acknowledgment on his behalf to the following persons for courtesies shown him and assistance rendered in his work: Mr. Austin Cary, of the Berlin Mills Company; Professor Harvey, of Orono, Me.; Mr. S. Boardman, of the Bangor Commercial; and Mr. Cram, of the Bangor and Aroostook Railroad.

Respectfully,

B. T. Galloway,
Chief of Division.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

Page.

Introduction 9
  Necessity for studying the diseases of forest trees 9
  Where the investigations reported were made 10
  Previous work on diseases of trees 11
Kinds of fungi growing on forest trees and their relation to forest problems... 11
  Extent of destruction 12
  External evidences of decay 12
  Relation to insect attacks 13
  Scope of this report 14
New England forests 15
  Vegetative conditions 15
  Red Spruce 15
  White Spruce 16
  Balsam Fir 17
  Hemlock 17
  Arbor Vitæ 17
  White Pine 18
  Tamarack 18
Polyporus schweinitzii Fr 18
  Occurrence 18
  Structure of diseased wood 19
  Fruiting organ 20
  Effect of fungus on the tree 23
  Trees attacked 24
  Methods of combating this fungus 24
Polyporus pinicola (Swartz) Fr 24
  Occurrence 24
  Structure of diseased wood 25
  Fruiting organ 29
Trametes pini (Brot.) Fr. forma *abietis* Karst 31
  Occurrence 31
  Destruction of spruce wood 32
  Destruction of fir wood 35
  Destruction of tamarack wood 35
  Fruiting organ 36
  Hymenium 40
Polyporus sulfureus (Bull.) Fr 40
  Occurrence 40
  Structure of diseased wood 40
  Minute changes in the wood 41
  Fruiting organ 42

Page.
Polyporus subacidus Peck 44
  Occurrence 44
  Structure of diseased wood 45
  Fruiting organ 48
  Remedies 49
Other diseases 49
  Polyporus vaporarius (Pers.) Fr 49
  Polyporus annosus Fr 49
  Agaricus melleus Vahl 50
Conclusion 51
Explanation of plates 53

ILLUSTRATIONS.

PLATES.

Page

PLATE I. Fig. 1.—Sporophores of *Polyporus schweinitzii* Fr. Fig. 2.—*Polyporus volvatus* Peck, growing from holes made in the bark by *Dendroctonus* sp 56

II. Log of Balsam Fir showing decay caused by *Polyporus schweinitzii* Fr. 56

III. Log of White Spruce showing early stage of decay caused by *Polyporus pinicola* (Swartz) Fr 56

IV. Log of White Spruce showing advanced stage of decay caused by *Polyporus pinicola* (Swartz) Fr 56

V. Sporophores of *Polyporus pinicola* (Swartz) Fr 56

VI. Fig. 1.—Red Spruce: Early stage of the decay caused by *Trametes pini* forma *abietis*. Fig. 2.—Red Spruce: Advanced stage of the decay caused by *Trametes pini* forma *abietis* 56

VII. Log of Balsam Fir showing decay caused by *Trametes pini* forma *abietis* 56

VIII. Fig. 1 early and fig. 2 late stage of decay of Larch caused by *Trametes pini* forma *abietis* 56

IX. *Polyporus subacidus* Pk., *Polyporus pinicola* (Swartz) Fr., and *Trametes pini* (Brot.) Fr. forma *abietis* Karst 56

X. Work of *Polyporus pinicola* (Swartz) Fr. and *Trametes pini* (Brot.) Fr. forma *abietis* Karst 56

XI. Stages of decay induced in Spruce by *Polyporus subacidus* Pk. and *Polyporus sulfureus* (Bull.) Fr 56

XII. Various forms of sporophores of *Trametes pini* (Brot.) Fr. forma *abietis* Karst 56

XIII. Block of White Spruce wood showing injury caused by *Polyporus sulfureus* 56

XIV. Fig. 1 early stage and figs. 2 and 3 successively later stages of the decay caused in White Spruce by *Polyporus subacidus* Peck 56

XV. Fig. 1.—Cross section of log of Spruce showing decay caused by *Polyporus subacidus* Peck. Fig. 2.—Resupinate form of sporophore of *Polyporus subacidus* Peck on Spruce log 56

TEXT FIGURES.

FIG. 1. *Polyporus schweinitzii* Fr. growing on a fallen Fir 21

2. Cross section of Spruce wood showing masses of mycelium of *Polyporus pinicola* (Swartz) Fr 28

3. Base of Spruce branch, showing its resistance to the attack of the mycelium of *Polyporus subacidus* Pk 47

SOME DISEASES OF NEW ENGLAND CONIFERS.

INTRODUCTION.

NECESSITY FOR STUDYING THE DISEASES OF FOREST TREES.

Very little attention has been paid to the study of diseases of forest trees in the United States up to this time, and the reasons are obvious enough. Up to within a few years the supply of standing timber of all kinds has been so large that a few diseased trees, more or less, scattered over wide areas were of little account. The lumberman cut down the sound trees and paid no attention to such as he recognized to be of inferior value. The situation has changed within the last decades, and a wide-felt demand has arisen among all classes of people for a more economical and rational treatment of the existing forest lands, and for reestablishing forests on denuded areas. In the primeval forest the trees diseased because of fungous or insect attack were ignored. They were few in comparison with sound trees, and the price of a single tree was very low. At the present time, with a marked appreciation in the value of timber, the agencies which injure trees for timber are of more immediate interest to the owners of woodlands. At this time the extent to which insects and fungi destroy trees can only be guessed at. Their work of destruction goes on silently here and there in the forest, and does not attract the attention of the casual observer as do careless lumbering or forest fires. If the dead and dying trees in a forest could be collected, they would represent a considerable percentage of the total forest. Forest fires are already not so common as they used to be, and the lumberman of to-day is beginning to understand that more can be realized from a given forest tract by rational treatment than by indiscriminate cutting. Insects and fungi, and other harmful agencies of less importance, are being studied with the aim of arriving at a more complete understanding of their manner of working.

From its first growth until it falls a tree is subject to attacks of a large number of insects and fungi, often resulting in stunted growth or death. In many cases the injury is to the wood alone; the diseased tree may remain standing for many years, and may be useful as a shade tree, but its value for timber has been destroyed. Besides the insects and fungi, diseases which may be characterized as physiological are not uncommon. They may be due to an insufficient supply of light, heat, water, or food, etc. Often insects and fungi act in conjunction with other unfavorable agencies, and it then becomes a matter of considerable difficulty to ascertain the true cause of the disease. The present paper deals only with diseases due to fungi.

The mycelia of fungi attack living trees as well as dead ones. When on living trees they grow either in the living parts, the roots, leaves, bark, or newer wood cells, or in the dead parts, the heartwood of the roots, trunk, and branches. The character of the injury which the mycelium causes depends much upon its place of growth, whether on the leaves or within the wood. Injury to the leaves may often be very great, as is the case with fungi like the *Erysipheæ*, *Uredineæ*, *Exoasceæ*, and others. The injury caused by those which grow in the living bark or cambium, like the species of *Nectria*, for instance, is very large. A large class of fungi flourishes within the heartwood of trees, growing into it through a branch or some wound, and in some cases through the roots. The effect of their growth is to destroy the heartwood, filling it with holes or turning it to a brittle substance which has none of the properties of ordinary wood. These changes weaken the trunk, and at some period or other the tree is broken by the wind. Those forms which enter through the roots may kill the latter first, and thus cause a tree to fall. The wood is then rapidly destroyed by a large variety of fungi and insects. It is therefore to the interest of the forester who grows trees for their wood to determine what fungi so affect the trees as to render the wood unfit for lumbering purposes.

In Europe, where forests have been grown for many years, the importance of understanding the diseases of forest trees has long been recognized, as is well shown by the works of Hartig, Tubeuf, Marshall Ward, Frank, Nypels, and others. These show that it is possible to prevent the growth of many of these fungi by destroying their fruiting bodies, and, in general, by bringing about conditions unfavorable to their growth and development. In order that this may be properly and successfully done, it is first necessary to know what the destructive fungi are and where and how they live. It was with this end in view that the writer spent several months during the year 1899 in the forests of Maine. A preliminary survey was made of the forests of that State, and the results are here presented in preliminary form.

WHERE THE INVESTIGATIONS REPORTED WERE MADE.

The region about Houlton in Aroostook County was first visited, then the territory north of Moosehead Lake, and during September the region about the Rangeley Lakes. A large part of the summer was spent on the coast at Linekin (near Boothbay Harbor, Maine), where the extensive spruce groves of both older and younger trees presented excellent opportunities for a study of the commoner forms. Collections of wood and fungi were made at all points visited. The basis of this report consists of field notes made in the regions visited, together with brief descriptions of the various forms of diseased wood. When the opportunities permitted, inoculations were made, by means of spores and mycelia, the results of which will not be apparent for many years.

PREVIOUS WORK ON DISEASES OF TREES.

Practically no work has so far been done on the diseases which affect the woody parts of the forest trees of the Northeastern States. Many descriptions have been published of the fungi which grow on these trees, but these deal mostly with the fruiting portion of these fungi and but rarely with the effects which they bring about in their substratum. Nearly all of the fungi of this class have been very thoroughly studied by Hartig[1] in Germany, and many of the conclusions of the present paper correspond with the results which he obtained. His studies, however, were confined to the effects of the fungi on the forest trees of Germany. The only notes on the forest fungi of America which the writer was able to find are those in Sargent's Silva of North America.[2] A number of the commoner fungi are there referred to briefly. The majority of these, however, are leaf fungi, viz, *Dasyscypha willkommii* R. Hartig, "said to occur in the United States, etc.," the various species of *Peridermium*, and a few others.

The catalogues of floras report many of the fungi herein noted, but the mere record of the occurrence of a fungus at one or more localities is of so little value in this connection that it was not considered worth while to present even an enumeration of them here.

KINDS OF FUNGI GROWING ON FOREST TREES AND THEIR RELATION TO FOREST PROBLEMS.

Of the fungi found growing on the wood of the coniferous trees but a small number bring about changes which completely destroy the wood. Many fungi grow on the bark of a dead tree or their mycelia penetrate into the living bark, where they flourish, but go no deeper. Others, again, grow in the bark and sapwood of trees after the latter have died, and in so doing destroy these parts. A third class grows in the heartwood only, or in heartwood, sapwood, and bark, whether the trees are alive or dead. Several of the last-mentioned class attack living trees and slowly bring about changes which ultimately result in death. Others grow within the heartwood, in which case the tree may remain alive as long as the trunk is strong enough to uphold the crown of branches. Some of these fungi can grow both as saprophytes, i. e., on dead wood, or as facultative parasites on living trees. In the following only those fungi are considered which so destroy the wood of the trees as to render it unfit for lumbering purposes.

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[1] Hartig, R. Zersetzungserscheinungen des Holzes der Nadelholzbäume und der Eiche. 1878.

[2] Sargent, C. S. Silva of North America. 12:5, 26. 1898.

The fungi to be described all belong to the *Polyporei.* Their sporophores form during the summer, and in several cases grow on during the winter months. They discharge their spores into the air in vast numbers, and these are carried to great distances by the wind. The spores germinate, under favorable conditions, in a wound or on the roots, and the mycelium makes its way into the inner parts of the tree, where it flourishes for a shorter or longer period, when the fruiting organ is again formed. The length of time which is required for the formation of the sporophores is variable, and is known for very few species. In some cases the sporophores grow only on the living trees; in other cases, again, they form for many years on the dead stumps or fallen trunks. Seasonal variations are to be met with. Some years, when it is exceptionally moist, the fruiting forms grow in great numbers, while during a dry summer very few are to be found.

EXTENT OF DESTRUCTION.

The amount of destruction which these fungi do is actually very large. As has been said, the casual observer does not note a dead tree here or there, but he is struck with the destruction wrought by forest fires. In certain localities the older trees are likely to become infected by one fungus or another, and it is a common saying of the lumberman that "the older trees are always rotten." If all the dead trees in a forest could be brought together, their number would truly be a surprise to lumbermen, the majority of whom have no appreciation of even the approximate destruction which is wrought in the forests in this way. Without extended cruisings it would be hazardous to make any more definite statement for the present than this: The number of dead and decayed trees is sufficiently large to represent a considerable loss in capital, and warrants making efforts to prevent the destruction of what would be valuable timber if harvested in time.

EXTERNAL EVIDENCES OF DECAY.

It is often a matter of considerable importance to recognize which trees in a forest have been attacked by fungi, so that these trees may be removed before they are completely destroyed and before there is any opportunity for the formation of sporophores. Trees which are in an advanced state of decay can usually be recognized by the fact that they have the fruiting organ of one or another fungus growing on their roots, trunks, or branches. The lumberman of the present day naturally tries to avoid trees which are rotted, and his method of diagnosis consists in pounding the trunk to see whether it sounds hollow. Hollowness, however, is not always a sign of disease, as many trees are hollow at the base and sound above, and therefore satisfy the demands of the lumberman at least in part. A test in use all over the country is the presence of what are variously known as punks, conchs, punk knots, resin knots, etc. A punk is usually the sporophore of *Trametes pini*, or some other large hoof-shaped sporophore. The other terms are more often applied to swellings which occur at points where a dead branch stub is found on the tree. In diseased trees of Pine or Spruce the turpentine is driven from the wood by the action of the mycelium of this or that fungus, and passes on before it, up the heartwood of old branches and out through them, forming resinous lumps, which harden in contact with the air. These lumps occur at all heights on the trunk and increase in size from year to year. The accumulation of these resinous masses prevents the normal healing of the wound or healing over of the stub of the branch, and results in the formation of a marked protuberance at that point, commonly called a knot, with its various modifications. The turpentine often drips from such a spot or runs down the bark in small streams. It may be many years before the decomposition within the tree advances sufficiently to enable sporophores to form, and a system of prophylactic treatment must take into account phenomena such as these to aid in detecting diseases in their early stages. What has been said with reference to these resin accumulations applies particularly to fungi like *Trametes pini* (Brot.) Fr. and its form *abietis* Karsten, to *Polyporus schweinitzii*, and *Polyporus sulfureus*, and one or two others not yet definitely identified.

RELATION TO INSECT ATTACKS.

The nature of the fungus injury is often very obscure, and there are so many factors which have to be considered in tracing the nature of any one disease that the results of the present paper are but fragmentary, and it is very probable that they will be modified largely by future discoveries. The intimate relationship which exists between the attacks of insects on the one hand and fungi on the other must be pointed out. There are without doubt many fungi which find their way into the wood of trees through the holes which boring insects have made in the bark. The injury which the insect makes may be very slight, but it has opened the way for the action of the fungus, which may be very destructive. An example of this kind is to be found in that most curious of all the *Polyporei*, *Polyporus volvatus* Peck. This grows on the trunks of spruces which have been attacked by various species of boring beetles, notably species of Dendroctonus. These beetles bore through the bark into the cambium layer. The fungus enters through these holes and grows in the sapwood of the tree, destroying it in a few months. Whether it grows there while the tree is still alive, and what its possible relations may be to the Dendroctonus, are problems yet to be solved. In many parts of the Maine woods every tree where the beetles had been or were still active was covered with the rounded fruiting organs of this Polyporus. (See Pl. I, fig. 2.) Their association with this Polyporus offers a promising field for study. The holes made by the beetles allow the spores of several other fungi to enter, notably those of *Polyporus pinicola*. These germinate and grow throughout the heartwood, rendering it worthless in a very short period.

The possible rôle which beetles and boring larvæ may play in carrying the spores of a fungus from one tree to another will be referred to below. These few instances will serve to show that it is all important that a study of the insect and fungus enemies of a tree should be made hand in hand.

There are grave inherent difficulties in determining the exact cause of death of a large tree, for there are many factors which may influence its growth so that the tree becomes weakened. There is a widespread opinion that insects or fungi will not attack an absolutely healthy tree, but that the latter must be more or less weakened before such an attack takes place. That this is not always the case need hardly be said, but the mere fact that a fungus is growing in the tree or an insect is at hand upon it is no positive proof that one or the other is the active agent in bringing about its death. Such evidence, particularly if oft repeated, will become very valuable when taken in conjunction with other proofs.

SCOPE OF THIS REPORT.

In the following a number of fungi will be described, together with the characteristic changes which their mycelia induce in the wood of the trees in which they grow. These fungi were found again and again, always associated with the forms of decay ascribed to them, and never was such decay found without the fungus in question, or without a mycelium from which the fruiting portion of the fungus developed. These fungi occurred on all coniferous forest trees, with few exceptions. Some of them started in the living trees and caused the heartwood to decay. They were found in large numbers destroying trees injured by insects, and on some tracts where fire had swept through the woods and had injured the bases of the tree trunks several of them had gained a foothold and had destroyed every tree thus injured. The principal ones met with were: *Polyporus schweinitzii* Fr.; *Polyporus pinicola* (Swartz) Fr., *Trametes pini* (Brot.) Fr. forma *abietis* Karsten; *Polyporus sulfureus* (Bull.) Fr.; and *Polyporus subacidus* Peck. A number of doubtful forms will be mentioned near the end of this report.

NEW ENGLAND FORESTS.

VEGETATIVE CONDITIONS.

The original forests of most of the New England States are gone. The White Pine, which at the advent of the white settler formed such a large part of the forests, is present in any large quantity only in the most inaccessible places and elsewhere as ripe timber only in isolated spots. The chief forest trees from the lumberman's standpoint are the Red Spruce and the White Spruce. Millions of feet of Red Spruce lumber are now being cut year after year in the States of Vermont, New Hampshire, and Maine. The time is not far distant, however, when the stand of spruce timber will be in a similar condition to that in which the White Pine is now.

The conditions which prevail in the forests of Maine and New Hampshire can be touched upon only in so far as they relate to the presence of and probable influence on the diseases which form the basis of this report. The forests are usually moist. The forest floor is covered with a large variety of mosses, which hold water very readily. Sphagnum covers many square miles. Springs and brooks are abundant everywhere. The annual rainfall, often very heavy during the spring and summer months, accounts for the general humidity of the air. Near the coast the fogs keep the woods moist for a large portion of the growing season. The summer season is usually comparatively short, but while it lasts very warm days are not uncommon. Warmth and humidity, chiefly the latter, are very influential in promoting the growth of many saprophytic as well as parasitic fungi.

Before describing the various fungi and their effects, it may be well to say something of the trees which are affected by these fungi.

RED SPRUCE.

Foremost among the coniferous trees of New England at the present time is the Red Spruce, *Picea rubens* Sarg. (*P. mariana* (Mill.) B. S. P., *P. nigra* Link). It is a tall, stately tree, which grows to be 70–80 feet (21–24 meters) high and 2–3 feet (0.6–1 meter) in diameter. It occurs all over northern New England, together with the Balsam Fir and White Pine. Sargent says of this tree:[1]

> Picea rubens, which is the principal timber spruce of the northeastern United States, and, with the exception of the White Pine, the most valuable coniferous timber tree of the region which it inhabits, produces light, soft, close-grained wood, which is not strong nor durable when exposed to the weather. It is pale, slightly tinged with red, with paler sapwood about two inches thick, and a satiny surface * * *. Now that the most valuable White Pine has been exhausted in the forests of the Northeastern States, the Red Spruce is their most important timber tree, and immense quantities of its lumber are manufactured every year from trees cut in Maine, New Hampshire, Vermont, and northern New York. * * *

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[1] Sargent, C. S. Silva of North America. 12:35. 1898.

The wood of the Red Spruce is used for construction, and thousands of trees of all sizes also find their way to the pulp mills for the manufacture of paper. During the summer of 1899 several large new mills were building in central Maine, one of which was expected to consume 300 tons of spruce wood daily. In a recent article in The Forester, Mr. Lyman, of the International Paper Company, discusses at length the use of this spruce for making pulp.

The tree is one of moderately slow growth. It reproduces itself well from seed, and grows up readily to replace the original stand of timber. In the forest, when growing in close stands, the lower branches die gradually and break off, leaving dead stubs which, in the case of larger branches, offer inviting spots for the entrance of fungus spores for several years after the fall of the dead branch. Attention has already been called by the writer to the manner in which different trees heal the wounds caused by dead branches.[1]

There are resin channels scattered through the summer wood. Their number varies considerably in the individual tree. In some trees there are but one or two in a given ring, while six or eight years later there may be two or three dozen.

WHITE SPRUCE.

The White Spruce, *Picea canadensis* (Mill.) B. S. P. (*P. alba* Link), a very much more stately tree than the Red Spruce, grows to a height of 150 feet (about 46 meters), with a trunk 3 to 4 feet (0.9 to 1.2 meters) in diameter. In the Northeastern States it is found in abundance, especially along the coast, and on some of the islands it is the only tree. It is widely distributed to the north and northwest, extending into Alaska. In the New England States it is not as abundant as the Red Spruce and is not used for lumber purposes to the same extent as its near relative.

> In the eastern provinces of Canada, where it is probably the only Spruce cut in large quantities, it is used in construction and for the interior finish of buildings and for paper pulp. * * * White Spruce lumber is also occasionally manufactured in Dakota and Montana, etc.[2] * * *

The wood of the White Spruce is straw yellow, very light, and not strong. Resin passages occur now and then in the very narrow band of summer cells. As an ornamental tree it is more extensively used than the Red Spruce.

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[1] Von Schrenk, H., Two Diseases of Red Cedar. Bul. No. 21, U. S. Dept. Agr., Div. Veg. Phys. and Path.

[2] Sargent, C. S. Silva of North America. **12**:37. 1898.

BALSAM FIR.

The Balsam Fir,[1] *Abies balsamea* (L.) Miller, is a tree common all over New England, springing up wherever the White Pine or Spruce are cut away. It produces great quantities of seed, which germinate readily the succeeding year. The trees are usually smaller than the Spruces, growing to be 50 feet (15 meters) in height and 6 inches to 1 foot (15 to 30 cm.) in diameter. Its wood is used for a cheap grade of lumber, for it is very light and does not have any resisting power. In central Maine it is often cut with the Spruce and sent to the pulp mills. The trees are very subject to the attacks of insects and fungi. The large black ants[2] annually destroy hundreds of trees.

HEMLOCK.

The Hemlock, *Tsuga canadensis* (L.) Carrière, is a stately tree, usually 60 feet (18 meters) in height, having a trunk 2 to 4 feet (0.6 to 1.2 meters) in diameter. It is an important element of the northern forest, and has long been valued for its bark, which is extensively used in the tanning of leather. As an ornamental tree it has few equals among our native trees.

> In stately grace it has no rival among the inhabitants of the gardens of the northern United States, when, with its long lower branches sweeping the lawn, it rises into a great pyramid, dark and somber in winter and light in early summer, with the tender yellow tones of its drooping branchlets and vernal foliage.[3]
>
> It is one of the most valuable trees of the Eastern forests. It is estimated that in the year 1887, 1,200,000 tons of bark of this tree were harvested, and although a large part of the timber of the trees cut and stripped of their bark is allowed to rot on the ground it is believed that the average annual value of the material of all kinds obtained from this hemlock is not less than $30,000,000.

The tree is one which grows very slowly. The seedlings are very sensitive to exposure and do not recover readily when injured. The wood is very coarse and brittle and is worked with difficulty. It is, however, used considerably in various localities for a cheap grade of lumber, and at times, when other wood is not to be had, for railway ties, fence posts, and railing, but its resisting powers to weathering influences are very slight.

ARBOR VITÆ.

The Arbor Vitæ, *Thuja occidentalis*, L.,[4] is a tree found throughout the northern parts of New England, particularly in wet, boggy lands, where it forms dense forests, the individual members of which grow to be 50 feet (15 meters) in height, with trunks 6 inches to 1 foot (15 to 30 cm.) in diameter. The wood is very durable and is on that account prized for fence posts and railway ties, for foundation walls, and for making shingles. The wood itself is rather coarse, yellow brown, and is free from resin ducts. The trees are grown as ornamental trees, particularly in the form of hedges.

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[1] Sargent, C. S. Silva of North America. 12:107, 108. 1898.

[2] Hopkins, A. D. Preliminary Report on the Insect Enemies of the Forests of the Northwest. Bul. No. 21, U. S. Dept. Agr., Div. Entomology. 1899.

[3] Sargent, loc. cit. 66.

[4] Sargent, C. S. Silva of North America. 10:126. 1896.

WHITE PINE.

The White Pine, *Pinus strobus* L., once so large a factor in the lumber industry of the New England States, is now comparatively rare as mature timber. It is subject to a number of diseases which will be treated of in a special paper. It is left out of consideration on that account in the present report.

TAMARACK.

The Larch, or Tamarack, *Larix laricina* (Du Roi) Koch (*L. americana* Michaux),[1] is a tall, stately forest tree which is found growing with the White Pine and Spruce and in some sections forms extensive forests, especially in low swampy lands. It grows throughout the Northern States, ranging from Maine westward to the western slopes of the Rocky Mountains, and southward to northern Pennsylvania, Indiana, and Illinois, and to central Minnesota. As an ornamental tree it is highly prized because of its graceful habit and thrifty growth. The wood of the Tamarack is extensively used in shipbuilding, for railway ties, and telegraph poles. It is very durable and hard. Compared with the White Oak, it has a crushing strength of 1.38. Dudley[2] says of it:

> The quality of the wood of this tree is such that it deserves to be widely known and more extensively used for ties than it has been. * * * The wood is easily treated with antiseptics to prevent decay, especially with sulphate or acetate of iron, and ties so treated have lasted over thirty years under heavy traffic.

POLYPORUS SCHWEINITZII Fr.

Polyporus schweinitzii Fr., Syst. **1**:351.
Polyporus schweinitzii Fr., Epic. 433.
Boletus sistotremoides Alb. and Schw., 243.

[Figured in Fries's Icon. Hym. No. 179.]

OCCURRENCE.

This fungus is one which is very common throughout the Northern forests on the Spruce and Fir, and, as Dr. Farlow remarks,[3] appears to be very much more prevalent in this country than in Europe.[4] It certainly stands near the top of the list in point of destructiveness.

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[1] Sargent, C. S. Silva of North America. **12**:7. 1898.

[2] Dudley, P. H. Bul. No. 1, Division of Forestry. Appendix I. 51.

[3] Sargent, C. S. Silva of North America. **11**:11. 1897.

[4] Hartig (Lehrbuch der Pflanzenkrankheiten. 177. 1900) says it occurs only on Pines.

It attacks young trees as well as older ones, entering the tree through the root system and growing up into the trunk for sometimes 40 and 50 feet (12 to 15 meters) from the ground. The mycelium makes the wood of the Spruce very brittle. Diseased wood is of a yellowish color; it has a cheesy consistency so that it can be cut across the grain with a knife quite readily and without much resistance. When dry, it is readily powdered. The brilliantly colored fruiting bodies are to be found in July and August growing about the base of the affected trees, more rarely on the trunks. (See text fig. 1; also Pl. I, fig 1.) It was found more frequently in places where the air was laden with moisture—for instance, along the coast and near lakes. On many of the islands which lie off the Maine coast the fungus was found to be very plentiful, even to a distance of 5 miles (8 kilometers) from the mainland, showing that the spores must be carried for a considerable distance. One small island had some 12 trees on it, all White Spruces, of which 7 had old fruiting organs of this fungus growing about the bases of the trunks.

STRUCTURE OF DISEASED WOOD.

The wood of the Red Spruce or the Fir when first invaded by the mycelium turns yellow, and after a time cracks here and there as if dried rapidly. A cross section of the trunk of a young Fir, made about 6 feet (about 2 meters) above the ground, is shown in Pl. II. The large crack at the side was made in chopping down the tree; the other cracks in the heartwood show plainly how the wood has shrunken. The structural changes which take place are as follows: Soon after the mycelium enters the wood of the Spruce the color changes and the wood becomes more or less brittle. This is due to the fact that at various points in the summer wood cracks appear in the walls of the tracheids and extend in the spiral direction around each tracheid. The break deepens gradually until it extends entirely through the secondary lamella up to the primary lamella. The latter remains unbroken. The spiral breaks increase in number and at last the tracheid has the appearance shown in Pl. IX, fig. 1. There appear to be two series of cracks, one extending upward from left to right, the other from right to left. This appearance is due to the fact, as Hartig has shown, that one sees the breaks in the walls of two tracheids at the same time. Hartig mentions that these cracks all extend in a spiral direction, none parallel to the walls. This is certainly a striking fact, and seems to distinguish wood attacked by this fungus from that injured by many others. It will be shown that some other fungi, *Polyporus sulfureus* and a form of *Polyporus destructor* possibly, attack the wood of the Spruce and the Yellow Pine, respectively, in a similar way.

The spring wood has few cracks. These are mainly in the pits, where four radiating cracks appear in the secondary lamella.

Wherever a hypha has passed through a wall is to be found the peculiar double spiral crack. (Pl. IX, fig. 1.)

The diseased wood of the Balsam Fir differs little from similarly affected Spruce wood. The summer tracheids as a rule are not so wide as those of the Spruce, hence the spiral cracks are not as evident as in that tree. They appear to extend more or less parallel with the walls. They are likewise present in greater numbers, so that there is very little left of the wall.

Wood which is in the last stages of decay is exceedingly brittle. It does not partake of the character of brown charcoal as much as does Pine wood similarly diseased, but is much firmer. It absorbs water very rapidly, and when boiled in water for a few minutes becomes soft and putty like and can be kneaded like bread dough. When dry it can not be cut with a knife without crumbling, but when soaked in water it can be readily cut into the thinnest sections. These have no elasticity, however. The walls of the wood cells are very thin and swell to several times their size on addition of dilute potash. Here and there are found masses of resin, more frequently in the Balsam Fir than in the Spruce. As the wood grows older the action of the mycelium seems to stop. The wood changes no further except that it cracks more or less. It appears to be very resistant to change brought about by weathering.

FRUITING ORGAN.

The first fruiting bodies observed began to appear toward the beginning of July. Small rounded masses grew out from the bark and very soon became flattened horizontally. The specimen shown in the photograph (fig. 1) was watched closely and measured daily from the time of its first appearance until it had reached its full size. By means of wires stuck at the edge of the growing shelves, it was easy to measure accurately the daily increase in diameter. The hyphæ rapidly grew around the wire so that it became embedded in the mass of the sporophore. One of the wires is visible at the right side of the middle shelf of fig. 1. The measurements show that for the first two weeks the larger shelves grew about one-fifth of an inch (one-half centimeter) a day in all directions; on warm days, however, the increase was more than that and on other days not so much. The youngest portion of the sporophore was yellow-brown, which in three or four days deepened to a red brown. The unequal development of the mass caused concentric rings to appear on the top of the pileus, showing by the low ridges and shallow furrows, respectively, where any particularly rapid growth had set in and where it had stopped. On August 15 the growth in width suddenly stopped. When full grown, the largest of the three shelves was 16 inches (40^{cm}) across at the widest point and 8 to 14 inches (20 to 35^{cm}) from front to back.

The sporophores grow either on the roots of an affected tree or on the trunk, the former being the usual position. When growing on the ground the pileus is supported on a very short stalk; it is sessile when growing from the trunk. There are usually several shelves which are grown together at the center in the ground form, or grown one above the other in the trunk form (see text figure 1 and Pl. I, fig. 1). The whole body varies greatly in size. The smallest specimens collected during the past summer were 4 inches (10^{cm}) in diameter; the largest about 14 inches (35^{cm}). The hymenial layer begins to form some three days after the body of the pileus is complete, so that there is always a wide band of sterile hyphæ on the under side of the pileus during the period that the pileus is growing in width. When this growth stops, the tubes gradually form close up to the edge. The hymenium when fresh is rose colored; when touched or bruised it turns dark red very quickly. The bright colors of the young pileus gradually give way to more subdued ones as the fungus grows older. A few days after growth has come to a standstill, the spores ripen and begin to be discharged. They come off in clouds plainly visible to the naked eye. Slips of glass placed under the pileus and left overnight had so thick a layer of spores deposited

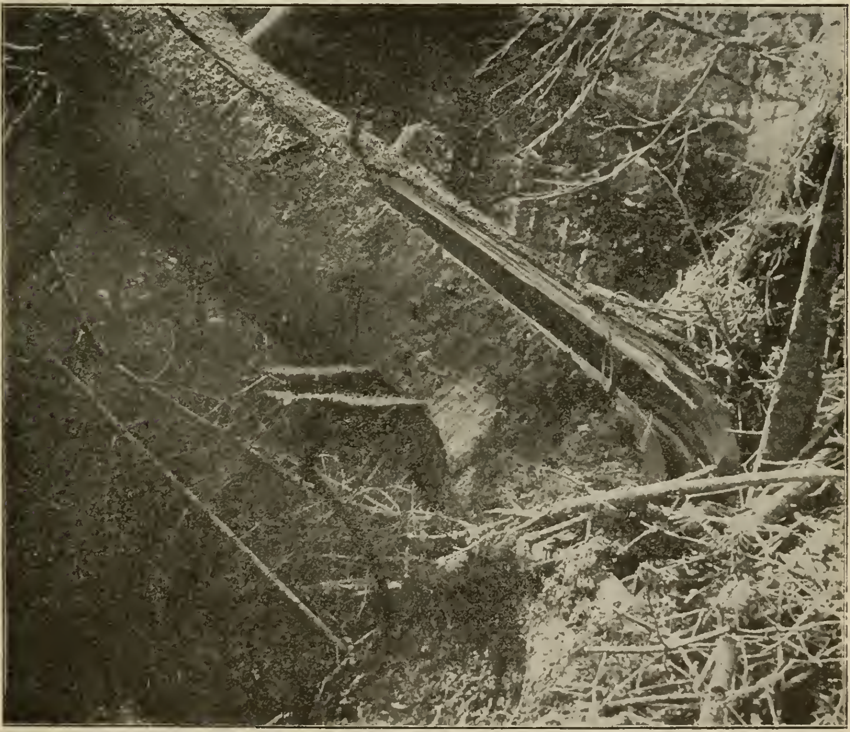

FIG. 1.—*Polyporus schweinitzii* Fr. growing on a fallen Fir.

on them that it was impossible to perceive anything through the glass. Attempts were made to grow the spores in the woods on bread cultures. These all failed, however, because of constant interference on the part of inquisitive squirrels.

The spores came off at intervals as if they were being discharged by some force acting within the tubes. Pieces of the pileus were accordingly turned over in a jar so that the tubes of the hymenium pointed up. Glass slips were supported over the tubes overnight, and on the following morning a few spores were found on them, but the number was so small when compared with the large number discharged from a similar piece laid with the pores pointing down, that it did not seem probable that there was any very active discharge going on. The spores were borne far away from the spot where they fell. Owing to their exceedingly small weight, every disturbance in the air carried them off. It was surprising to see how slight a disturbance sufficed. The flame of a candle held near the sporophore remained perfectly motionless while clouds of spores were swaying to and fro under the hymenial layers. The spores were sown in aqueous decoctions of humus, but did not germinate. The facilities for doing more careful work were not at hand in the woods, so experiments on the manner of germination had to be left for a future time.

At the time of ripening of the spores it was noticed that hundreds of drops of a yellowish liquid were hanging from the hymenial surfaces every morning when the fungus in question was visited. Some of these drops were carefully collected and were examined. In them floated a number of spores and flocculent yellowish brown masses, which stained yellow with nitric acid. These were present for several days. Thereafter the liquid was almost clear except for numberless spores which were in every drop. For three weeks the drops were collected with a pipette during the day, and during the night a plate, carefully protected against dew and rain, was placed under the fungus. In this way about three-fifths of a pint (300^{cc}) of liquid were collected. This was poured into an open dish and put in a cool place, where the water was allowed to evaporate. A thick brown sirup was left after some weeks, which had the odor of very impure molasses. The sirup was transferred to a vial, which was corked and placed in a warm place. In a few days delicate needle-shaped crystals shot out, which upon examination proved to be melezitose and mycose, sugars sometimes found in fungi.[1]

At the same time that this secretion appeared on the hymenium, or rather shortly afterwards, a number of small beetles began to devour the hymenium with great avidity. So active were they that within three weeks of their appearance the hymenium was entirely destroyed, and of course with it whatever spores had remained. It is suggested that the secretion of this sugar and the destruction of the hymenium by these beetles may have some meaning in connection with the dispersal of the spores. It is a point worthy of further observations by local observers in future years. The rapid destruction of the hymenium is very marked. It is exceedingly difficult to get perfect specimens of the sporophores after the end of August. The upper surface, which is usually moist, becomes covered with a fine layer of fallen spruce needles, and before long a covering of mosses hides the brown sporophore completely. It is no unusual occurrence to find these old moss-covered sporophores several years after their formation, at the base of some old Spruce.

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[1] The writer is indebted to Dr. O. Loew for the determination of these sugars.

The basidia and spores have nothing about them which is very distinctive. Numerous peculiar hairs project from the hymenium, which are surrounded with a film or drop of clear liquid in which numerous spores are caught. When viewed by reflected light these glisten like dewdrops within the pores. The latter are exceedingly irregular, so irregular in fact that one can hardly call them pores. They partake more of the nature of pockets, which are divided by many much convoluted walls into various chambers. The pores extend almost to the margin of the pileus and are usually about one-eighth of an inch (3^{mm}) deep.

EFFECT OF FUNGUS ON THE TREE.

The fungus seems to spread through the ground, attacking the tree first at its root system, and growing thence up into the trunk. Wherever one tree is affected, others similarly diseased will usually be found close by. Infection may take place through the root on one side of a tree. The heartwood of that root will be destroyed and then the wood of the portion of the trunk nearest that root becomes affected. Many trees were cut down where but one-half of the trunk had been rotted by the fungus, and oftentimes only a small spot was visible where the fungus had just begun to grow. The tree continues to stand until either the roots or the trunk become weakened to such an extent that they can no longer hold the tree erect, and then the first wind storm overturns it. Fig. 1 shows a large Fir, the root system of which was almost entirely destroyed. In its fall the lower part of the trunk split, revealing decayed wood to a distance of 12 feet (about 3⅔ meters). The tree was probably blown over in the spring of 1899, and in the following July the sporophores formed on the trunk. A large tree thus diseased is a constant source of danger to all others about it. Not only may the disease be communicated to them, but in its fall such a tree breaks down many a small tree, not to speak of the large numbers of very small second growth which it destroys. The sporophores form on fallen trees for several years in succession, possibly omitting a year now and then. As a rule but one set of sporophores is found on one tree. As has already been said, young trees are subject to the attacks of this fungus as well as older ones, although the latter are probably more so, because the points of infection are so much more numerous. Nothing is known as yet of the manner in which the fungus enters the tree, nor of the rate at which it grows within a tree after having obtained a foothold.

TREES ATTACKED.

Polyporus schweinitzii was found growing on the roots of the White and Red Spruces, Balsam Fir, and Arbor Vitæ. It is likewise common on the White Pine (*Pinus strobus*).

METHODS OF COMBATING THIS FUNGUS.

Because of its destructiveness *Polyporus schweinitzii* is perhaps the most to be feared, where living trees are concerned. As it spreads through the soil it is difficult to detect, and still more difficult to combat. In the European forests a deep trench is dug around an infected tree or group of trees; this trench prevents the spread of the mycelium through the ground to neighboring trees. Such a method can not be recommended for American forest-tree conditions, at least not for the present. If a group of infected trees is met with in the forest while lumbering it may prove advantageous to cut all trees in the vicinity of the diseased ones. Some of these may produce a hollow sound when hit near the base, an indication that the decay has started. It may not have gone up into the tree very far as yet, so that one or more logs can be obtained from the top. It will not be profitable to hunt out diseased trees as is done in European forests. There is as yet no evidence that the fungus can infect a tree above ground, consequently it need not be feared in burned-over regions, or such as have been attacked by bark beetles.

POLYPORUS PINICOLA (Swartz) Fr.

OCCURRENCE.

This fungus occurs widely distributed over the world, growing on conifers and occasionally on Birches and other deciduous trees. In the New England forests it is one of the most frequent fungi found on living or more often on dead trees of Spruce, Pine, Fir, and Hemlock. From three to ten of its bright colored sporophores may grow on a single log for several, varying from three to five, years. At the end of that time the mycelium has used up the available food supply in the log and dies.

The sporophores grow on living trees, but these always appear weakened or sickly. No vigorous, healthy trees were found on which this fungus flourished. It is essentially a wound parasite, entering the trunks and branches above ground. Old knot holes or branch wounds, wounds produced by fire, or wounds made by animals, are favorable spots for the entrance of the spores. Wherever a tree dies from any cause this fungus is sure to attack it before long. In the sections where the bark beetles had been active some years ago there were many trees the wood of which had been destroyed by this Polyporus.

The large holes made by woodpeckers offer excellent opportunities for the entrance of spores. As the woodpeckers are very active in exterminating insects inhabiting the bark (presumably the bark beetles among others), we have here a case of their allowing one enemy to enter while destroying another. In old windfalls the dead trees were covered with sporophores, some of them many years old, showing that these trees had become infected very soon after the trees had been blown over. This fact is of importance, as it suggests that these trees could be saved by the lumberman if carried to the mills shortly after their downfall. This will be referred to again. Plate IV is from a photograph of a portion of a Spruce trunk. The small white spots in the bark are holes of a borer filled with the mycelium of the fungus.

STRUCTURE OF DISEASED WOOD.

Wood of the Spruce in which the mycelium of this Polyporus has been growing for some time deserves the description "entirely rotted" par excellence. The wood has been changed to a brittle red-brown mass, which has cracked in many directions. The individual pieces are barely held together by countless sheets of mycelium which have filled the spaces resulting from the cracking of the wood and form an intricate network of larger and smaller sheets. In Pl. IV a portion of a log in the last stages of decay is shown. At one side a sporophore one year old and another just beginning are visible. The sapwood has numerous tunnels of a borer filled with remnants of the borings. Such wood has lost all strength, and falls to pieces at the slightest touch. If the mycelium attacks a standing tree the decay goes on within it until the trunk becomes so weak that an ordinary wind blows it over. The shrinkage which takes place in the wood as it is being metamorphosed is very considerable, as is evidenced by the large number of wide cracks which fill it, passing both across the annual rings and parallel to them.

The changes which result in the wood may be described as follows: In a tree just attacked the wood about the point of entrance of the fungus turns darker and finally becomes a decided red-brown. Before long small whitish areas appear here and there scattered irregularly through the wood. Some of these are mere lines, while others form white patches circular in shape, surrounding small areas of wood about the size of a pinhead, which are red-brown (Pl. III). Others again have the shape of broad, irregular bands, which extend across the rings of growth. The points at which these white areas appear and the direction which they take do not seem to be controlled by any particular factor, for they are exceedingly irregular. The areas are shown in Pl. III, which represents a radial view of a spruce log in the early stages of attack by the mycelium. The very fine white lines which are visible near the center of the log, extending across the annual rings, are of a different character from the white areas spoken of. It will be noted that in the white areas the parallel lines which indicate the summer wood are very distinct. A microscopic examination of a white area shows that at this point the cells of the wood are completely filled with fine hyphæ, which form a dense mass within that area. Mixed in with the mycelium are the granules of an amorphous substance, readily soluble in alcohol, which is evidently resin. This, together with the mycelium, gives the white appearance to the spots. As the summer tracheids have a very small lumen, they have comparatively little mycelium, which accounts for their being visible as lines extending through the areas. The size of an area is thus dependent upon the distance to which the mycelium has grown, and probably varies from time to time. It is suggested that the smaller areas are also the ones most recently invaded. At this stage of the decomposition the mass of the wood is already very brittle. Here and there cracks have appeared in the walls of the wood cells wherever a hypha has passed through them. Some tracheids appear like sieves because of the numerous holes. The changes subsequent to this stage of decomposition consist essentially in a carbonizing of the wood and the formation of the sheets of mycelium. The former change is one probably induced by some ferment, the nature of which it is the intention to discuss more fully in another report. The cells of the wood gradually show more and more cracks and fissures, and the diameter of the walls decreases about half. The main shrinkage takes place in the secondary lamellæ. The fissures which appear in the spring wood usually emanate from the holes formed by hyphæ. The outline of these holes is irregular, but approaches a circle in form. In the secondary wall a fissure is soon formed which extends diagonally from left to right across the cell. Viewed from the top there are apparently two fissures, but these can readily be shown to belong to the secondary lamellæ of adjoining cells. The fissures never extend into the primary lamellæ. Various stages of such fissures are shown at Pl. X, fig. 4. In the bordered pits at first two and later four fissures are visible in the secondary ring, which, as Hartig has surmised,[1] are probably brought about by drying. Here and there (Pl. X, fig. 4, c) a hypha has passed directly through a bordered pit and in its passage has dissolved more or less of the membranes. In the summer tracheids the number of fissures in the walls is very large. They all extend diagonally across the tracheids as in the spring cells, and wherever a hole occurs there two fissures seem to cross. The smaller fissures have no counterpart in the secondary lamellæ of neighboring cells as a rule, showing the complete independence of the two halves of the cell wall. The margins of the fissures are ragged, and the fissures themselves are very irregular in shape, and look as if they had been formed suddenly. The wood substance itself has been changed completely. With phloroglucin and hydrochloric acid it stains red, and when the wood, finely powdered, is extracted with absolute alcohol quantities of hadromal are obtained. No reaction for cellulose can be obtained, and it seems as if the latter has been completely destroyed. An aqueous solution yields several compounds—an amorphous substance, possibly a humus compound; a faint trace of some sugar, as shown by the phenylhydrazine test; and small quantities of citric and succinic acids. These are doubtless all decomposition products. There is also some compound present which reduces Fehling's solution vigorously. After the walls of the tracheids are filled with holes and fissures they have reached the last stage of decomposition. A touch will then cause them to fall into many pieces. When boiled in water, the walls swell somewhat, and a pasty mass results when squeezed. With dilute KOH the walls swell to three times their size, and portions of them dissolve completely.

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[1] Hartig, Robert. Zersetzungserscheinungen des Holzes, etc.

The formation of the sheets of mycelium seems to take place in one of two ways. In one case the hyphæ in the white areas mentioned above exert a solvent action on the walls. This first becomes evident in the cells of the medullary rays. Their walls disappear completely, and the spaces are rapidly filled by the growing mycelium. The walls of the wood cells adjoining the medullary rays are attacked, possibly at the same time. The secondary lamellæ shrink and finally disappear altogether, leaving a fine framework of the primary lamella. This framework is usually broken in hundreds of places, and as a result only pieces of the walls remain embedded in a web of hyphæ. The bordered pits are destroyed from within outward, the torus resisting longest (Pl. X, fig. 5). The latter is often freed and can be seen lying free in the cell. The triangular areas (as seen in cross section) of the primary lamella, formed where several cells join, are the last to disappear. A hole is thus formed which is completely filled with mycelium. The latter spreads from this point in several directions. The writer is of the opinion that very little actual solution of the wood takes place, resulting in the formation of holes or cavities. Here and there it doubtless does occur, but rather as the exception, and possibly in the early stages of the development of the mycelium. It seems very much more probable that the second mode is the usual one. As the mycelium spreads through the wood it brings about the chemical changes spoken of, extracting substances from the walls. This reduces the volume of the wood and causes the fissures in the cells. But before long the shrinkage becomes so great that larger masses of the wood suddenly break away from each other at many points throughout the wood. Many small fissures are thus formed, which extend in every direction, both across the rings and within them in a tangential direction. The fissures are very irregular. Sometimes they extend for a short distance within one ring, then cross over into another, and so on. They appear both in the spring and summer wood, and not infrequently start in one ring and extend radially through the summer wood of that ring into the spring wood of the next. Often the breaks follow the lines of the medullary rays, but just as often they do not (Pl. X, fig. 1). The process is evidently one of drying, for the same result is seen when wood dries, resulting in the formation of fissures, the so-called "checking" of wood. If the fissures are near points where mycelium of the fungus flourishes, the latter grows into the spaces and fills them completely. Several fissures may join, forming an irregular longer one. In Pl. X, fig. 1, a sketch is shown of the cross section of several annual rings, showing how and where the breaks have formed. As the wood dries more and more the fissures widen and the mycelium keeps step with them. In small fissures it is very evident that the fissure has formed as a break and not by the solvent action of the mycelium. Fig. 2 shows such a fissure filled with mycelium. (The same figure is shown at *c*, Pl. X, fig. 1.) A glance at the rows of wood cells will show how they have been forced apart, breaking one row. The rows are inclined toward one another, as one would expect them to be. The figure also shows a medullary ray at the right, the walls of which have disappeared. In the cells surrounding the break the mycelium flourishes, and here and there some of the walls are destroyed, making a small hole.

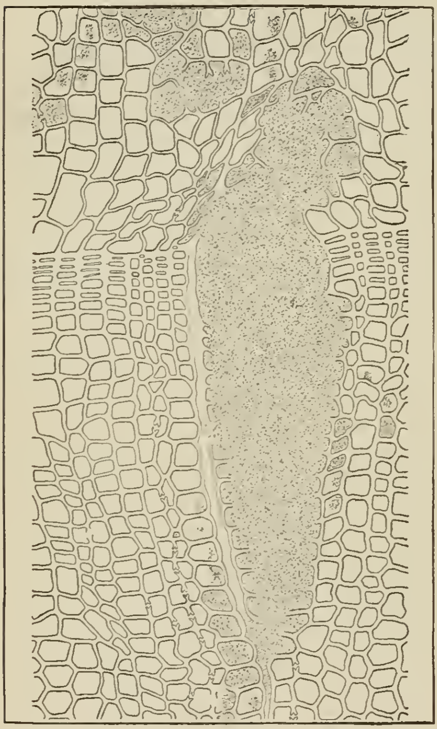

FIG. 2.—Cross section of Spruce wood showing masses of mycelium of *Polyporus pinicola* (Swartz) Fr.

In the last stages of decay the fissures are very numerous, each filled with a solid felt of white mycelium. The felts extending in radial lines join those extending in tangential lines here and there, and they hold in place the wood which would otherwise have fallen to pieces long before. In a live tree the heartwood is attacked first, and gradually the decay spreads to the sapwood. In the latter the browning of the wood is more marked, owing to its lighter color. Nothing positive can be stated at present as to the rapidity with which the decay brought about by this fungus proceeds. It appears to be very rapid, for trees blown down some two years before were found in an advanced stage of decomposition, with sporophores forming on their trunks at various places.

FRUITING ORGAN.

The sporophores of this fungus are very large and conspicuous, and are formed on logs during spring and summer, often many together on the same log. The form of the pileus is exceedingly variable (Pl. V). It is entirely resupinate when growing on the lower side of an overturned log (Pl. IX, fig. 6). In such a case there is no upper surface for several years. After three or four years the edge extends out beyond the curved surface of the log, and a narrow surface is exposed. Usually the pileus forms a distinct bracket on the side of a standing trunk or log. This bracket is sometimes hoof-shaped, then again very much extended. In size it varies from an inch to a foot (2.5 to 30^{cm}) in width, or even more in extreme cases. The average specimen is 4 to 6 inches (10 to 15^{cm}) wide. The upper surface of the bracket slopes toward the margin, and is divided into a number of regular divisions or lobes, which correspond evidently to periods of growth (Pl. V). The lobes are smooth and dark red-brown when old. The youngest lobe is bright red, shading into a pale yellow at the very edge of the pileus. In many specimens the upper surface is almost black, and some of the lobes shine as if varnished. The number of lobes varies with the age of the specimen; one of the oldest found had fourteen. It has not been determined whether these lobes represent annual increments of growth, so it is not possible to say how old any of these large sporophores may be. The mass of the pileus is extremely hard and woody, and shows division into a number of zones (Pl. IX, figs. 5, 6, 7), which are always greater in number than the lobes showing at the top. The hymenium is a pale yellow, very smooth, and assumes a watery appearance when bruised. It is very rarely perfect, as many insects are constantly at work eating away the tissue. The outer edge of the lower surface of the pileus is raised, forming a distinct ridge. At the inner edge of this ridge the formation of the tubes of the hymenium begins. This ridge is continuous around the whole lower surface and forms a character which is very constant. In young individuals it is wider than in older ones. It is composed of loosely interwoven hyphæ, which form a continuation of the main hyphal strands which compose the body of the pileus. The hyphæ of the latter start from a central point on the bark and radiate out in several directions (Pl. IX, fig. 7), forming a mesh which at first is very loose. The hyphæ are almost colorless and have a decided lumen. As they grow older their walls become brown and very thick, so that the lumen is reduced to a very small one. The peripheral growth of the hyphæ takes place in such a way as to form well-defined layers. For several years these layers are added one outside of the other. The lowermost portion of each layer is usually less dense than the outer portion, and after the hyphæ turn brown large masses of crystals of calcium oxalate are deposited in the meshes of the outer portion. The alternation of layers of less density with those of greater density makes a differentiation of layers possible. The layers vary considerably in width (Pl. IX, figs. 5–7), and it is suggested that this is probably due to varying conditions; probably the amount of food supplied and the amount of available moisture exert a marked influence. The pileus grows in width and length by the direct elongation of the hyphæ of the last layer. After several years' growth the hyphæ on the under side of the developing shelf grow down in a vertical direction and give rise to the pores.

The pores are very long and are continuous from year to year. After a time they become plugged at the bottom by hyphæ which grow into them from all sides. Different sporophores differ in this respect. With some the pores are open through eight or ten of the recent layers; in others the growth of hyphæ is so vigorous that the pores are closed almost as rapidly as they are formed. The hymenium arises on the surfaces of the pores from hyphæ of the trama which turn at right angles to the general direction of the tramal hyphæ. The latter have very thick walls (Pl. IX, fig. 12) and extend longitudinally, forming a very loose network. The tips of those hyphæ which form the hymenial layer are thin walled. The hymenial layer itself is composed of hyphæ of almost equal width. The layer is a very narrow one. Cystidia are practically absent. The basidia barely rise above the general surface and do not differ materially in form from the paraphyses. The four spores are colorless. Amid the tramal and hymenial hyphæ accumulations of calcium oxalate crystals, colored red-brown, occur in great numbers, likewise large quantities of an oil readily soluble in ether and becoming solid at about 59° F. (15° C.). The growth of the hymenial layer is very irregular. At one and the same time pores may be forming on one side, while at the opposite side the old pores are completely plugged. The hymenium renews itself at frequent intervals. The vitality of its hyphæ is very great, for it is not at all rare that insects eat away a considerable portion of the lower side of the pileus. These parts die and turn brown.

The remaining portions then form separate centers of growth, which gradually spread over the dead portion and unite, after several years perhaps, completely covering the dead part. A view of such a pileus is shown on Pl. IX, fig. 4; several areas have already joined, forming a larger one, and a number of small centers are evident.

The spores begin to be discharged in July. Growth of the lower side of the pileus takes place at the same time. Black cloths were pinned to the under side in June and by the end of August large portions of them were found completely overgrown with hyphæ, and pores were beginning to form on the under side of the cloth. While the growing season lasts drops of a glistening yellow liquid are constantly being discharged from the hymenium. It is of interest to note here that the secretion of these drops was noticed by Fries in a description of this fungus.[1] Several cubic centimeters of these were collected and were found to hold in solution melezitose, the same sugar discharged from the sporophores of *Polyporus schweinitzii.* As insects, particularly small boring beetles, eat the hymenium with great avidity, it is possible that the sugar may serve to attract these insects to the sporophores, causing them to carry the spores to uninfected trees.

TRAMETES PINI (**Brot.**) **Fr. forma ABIETIS Karst.**

Polyporus piceinus Peck.
Polyporus abietis Karsten.

OCCURRENCE.

This fungus is very common in the forests of the New England States, and occurs northward into Canada and Newfoundland. The writer found it common on the Spruces and Firs in the Adirondack forests. It grows on nearly all the conifers and has been found by the writer on the White Pine (*Pinus strobus*), the Red Spruce (*Picea rubens*), the White Spruce (*Picea canadensis*), the Hemlock (*Tsuga canadensis*), the Larch, or Tamarack (*Larix laricina*), and the Fir (*Abies balsamea*). It attacks living trees after they have reached such a size that they form heartwood, and honeycombs the wood in such a way that it appears filled with small holes, many of which are coated with a shining white lining. The changes which are brought about in the wood are different somewhat for the different kinds of trees and will be described separately. Of the six trees the Tamarack seems to be the most readily attacked. A greater per cent of the older trees of this species were found affected than of the other five. The Spruces came next, and the Balsam Fir last.

The fungus enters the trees through the stubs of broken branches and through wounds. The mycelium flourishes in both heartwood and sapwood of the Spruces, the Fir, and Tamarack, and is confined to the heartwood in the Pine. It grows up and down the trunk from the point of infection, reaching into the root system and extending into the larger branches of the top. Affected trees may remain standing in the forest for many years until some more violent storm breaks the trunk at a weak point. The wood of the trunk is never destroyed completely, as in the case of the two fungi described above. In the most advanced stages of decay some fibers of unchanged wood are to be found. The extent of their presence varies with the tree.

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[1] Fries, Elias. Epicrisis Syst. Myc. 468. 1836–1838.

DESTRUCTION OF SPRUCE WOOD.

The first effect noticed when the mycelium grows in the wood of either of the Spruces is a change in color from the light straw yellow of the normal wood to a light purplish gray closely approaching the color indicated on the Milton Bradley Color Scale as Neutral Gray No. 1. Very soon this gray deepens to a red brown, the gray remaining as an outer ring surrounding the portions of red-brown wood. Small black lines appear scattered here and there through the red wood. These lines are present throughout an annual ring and extend longitudinally in the direction of the wood fibers for a distance of $\frac{1}{50}$ to $\frac{1}{25}$ of an inch (0.5 to 1 millimeter). Gradually the black lines disappear and here and there small white areas appear (Pl. VI, fig. 1). The central portion of each area is absorbed and small holes are formed, which have white linings of loose fibers. The holes are at some distance from one another and are generally arranged in rows corresponding to the annual rings. Where the latter are very wide there may be a row of holes in each ring. The holes generally have their centers within the summer wood of the annual ring, but as they increase in size portions of the spring wood of that particular ring, as well as the spring wood of the following ring, are included. The holes have a more or less spherical shape, which soon changes to a more or less elongated form, the greatest diameter extending radially. Pl. X, fig. 2, shows a cross section of a piece of wood at an early stage of the destruction. Some of the holes at this period are filled with a mass of white fibers, so that there is practically no hole. The outlines shown in fig. 2 of Pl. X represent the outer limiting line of the white fibers, and the dotted lines (where present) indicate where the actual cavity begins. As the growth of the mycelium progresses, the holes increase in size and their walls approach one another until only a narrow lamella is left (Pl. X, fig. 3).

A large number of holes appear between the original ones, and in the final stages there is practically no wood left except the narrow walls separating two holes (Pl. X, fig. 3, and Pl. VI, fig. 2). Adjoining cavities rarely, if ever, unite to form a larger one in a lateral direction. They often unite at their upper and lower ends, forming a longer hole. The holes are never sharply defined, for there is always more or less of a white mass of metamorphosed fibers which remain in position next to the unchanged wood, and in many cases the whole area is thus occupied, and one can recognize the change only by the white color. In older holes this lining is often replaced by felts of brown mycelium (Pl. X, fig. 3) which partially or completely fill the cavity. The lamellæ of wood between the holes ultimately become of an almost uniform thickness (Pl. X, fig. 3), and on cross section show one or more black lines which extend completely around each cavity at an equal distance from the walls of two adjoining cavities. These black lines begin to appear at a stage intermediate between that shown in fig. 2 and fig. 3 of Pl. X. They are of variable width and grow darker and more marked as the decomposition advances. A longitudinal section shows that they extend around the holes in a vertical direction also; in other words, a thin layer of dark-brown matter surrounds the individual cavities. A closer examination shows that the brown lines are due to masses of dark-brown hyphæ which fill each separate wood cell so completely as to plug it entirely. The hyphæ are closely matted together and are incrusted with a brown substance which dissolves in part in dilute KOH and entirely in warm nitric acid. These hyphal plugs occur in every tracheid surrounding a hole and fill it for a shorter or longer distance. The plugs of adjacent cells may be continuous, or may follow one another much as a series of steps. This is shown in Pl. IX, figs. 10 and 13. The latter represents a radial view of a number of tracheids at one side of a hole. The parts of the tracheids toward the hole (*t*) are completely changed to white cellulose fibers, while the parts on the other side of the plug (*l*) give lignin reaction. The brown hyphæ fill the wood between the holes rather loosely, and it is only when about half way between two cavities that they become matted together so as to form the plugs. The brown incrusting substances occur in or on the cell walls in the immediate neighborhood of the holes, and the manner of occurrence leads one to suspect that they were deposited in liquid form, for they have diffused through the various cells in all directions from the wall of the cavities.

The changes in the cell walls which result when the mycelium attacks them are practically those so fully described by Hartig.[1] There is a gradual extraction of those elements which give the so-called lignin reaction, the hadromal of Czapek. This begins in the tertiary lamella and proceeds outward slowly through the secondary lamella. The primary lamella at this period splits in the middle and is shortly after dissolved, leaving the individual tracheids entirely free from one another, each composed of approximately pure cellulose.

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[1] Hartig, Robert. Zersetzungserscheinungen des Holzes, etc. 32.

The parts of the primary lamella which are situated between three or more cells resist longest (Pl. IX, fig. 9, *p*) and can be found free between the white cellulose fibers. The change to cellulose apparently takes place simultaneously over a considerable area. The first evidence of this change is to be seen in the white spots which come after the black lines. The white spots are the points at which the change to cellulose has taken place. The cellulose fibers are absorbed later on, giving rise to the holes already mentioned. Preceding the change from wood fiber to cellulose the wood is full of hyphæ, which become massed in centers here and there and bring about the dissolution of the wood. It is as yet undetermined what causes influence this local initiation of the changes, which is characteristic of several other wood-destroying fungi. The growth in size of the white spots or cavities takes place rapidly. The hyphæ grow out in all directions from the original center, and as they do so the products of decomposition pass outward likewise, passing along the tracheids faster than across them. After a period the advancing hyphal masses of two adjacent holes meet in the narrow lamella of unchanged wood between the two. A quantity of brown substance, representing decomposition products, has by this time accumulated. It fills the tracheids and coats the hyphæ so that these turn very dark, almost black. Warm nitric acid removes these substances entirely, leaving the hyphæ and wood almost colorless. It is the opinion of the writer that this accumulation of the products of decomposition may account for the fact that the destruction of the wood stops at this point, thus preventing the total destruction of the wood substance. That this can not be true in all cases is shown by the fact that many of the cavities join in the direction of the fibers, but in this instance it is probable that diffusion takes place to more remote places. The mass of cellulose within the affected areas consists of free fibers which remain in place for a period and are then gradually dissolved here and there, leaving an actual hole with a lining of white fibers.

In the newly invaded parts of a trunk the mycelium is colorless and fills the tracheids completely. The individual hyphæ are somewhat thick-walled and have numerous short branches which penetrate the cell walls in all directions, leaving the characteristic figure 8 holes described by Hartig and others.

Here and there a second form of decomposition occurs in which there is no reduction to cellulose. The process, as found in the spruce, is essentially the same as described by Hartig. The secondary lamellæ are gradually absorbed, leaving the primary lamella intact. The wood gradually changes into a mass of red-brown fibers which fall apart at the slightest touch.

The destruction of the wood takes place throughout the trunk, including the heart and sapwood, and finally even the bark (see Pl.

VI). A trunk like that from which the log shown in Pl. VI, fig. 2, was taken decays no further, and may stand in the forest for many years. After a tree has once fallen the destruction seems to stop. Two trees under observation for more than a year did not change at all. In both the decomposition had reached, in 1898, the stage shown in fig. 2, Pl. VI, and in September, 1899, no further change could be detected. Further observations in this connection are desirable. This point is perhaps not as important from the standpoint of the forester as the power of the fungus to form fruiting organs after the fall of a tree, and this assuredly takes place with this fungus for several years, as will be mentioned.

DESTRUCTION OF FIR WOOD.

The destruction of the wood of Balsam Fir, *Abies balsamea*, does not differ materially from that of the Spruce. White spots appear in newly attacked wood, which soon grow into larger ones; the black lines surround the individual holes sooner or later and then the decay ceases. On Pl. VII a radial view is shown of a log taken from a Fir which had been blown down during the past summer.

DESTRUCTION OF TAMARACK WOOD.

The process of destruction is very different in the Tamarack. This is probably due to the different nature of the wood of this tree, which seems to be far less resistant than the others. In the Tamarack the decay goes much beyond that described for the Spruce and Fir. In the early stages (Pl. VIII, fig. 1) small white spots appear, which usually occupy the entire width of an annual ring. Two or more of these spots soon join, at first in a longitudinal direction, then laterally also. In that way it happens that very early in the process of destruction long stretches of one or more rings of wood are transformed to cellulose. This is well shown in fig. 1 of Pl. VIII. This brings about the separation of one or more rings from the adjoining ones, forming in that way a series of tangential plates which can readily be separated. In the figure each one of the plates visible at the upper end represents one annual ring. The line of separation between the rings is always at the point where the summer wood stops and the spring wood of the following year begins. As the decay continues, more and more of the sound wood fibers are attacked, leaving loose cellulose fibers. When most of the wood has disappeared, black lines similar to those described for the Spruce appear, but as there are no such centers of decay as in that tree the lines are scattered irregularly. It would seem as if there were few decomposition products formed in the Tamarack, and then only at a very late date. Ultimately the tangential plates become extremely thin; they are then composed of the more resistant summer wood cells of this or that wood ring, which are more or less infiltrated with resin. The whole body of the former wood is a mass of separate fibers, which can be pulled out individually. This can be seen at the ends of the piece of wood shown in fig. 2 of Pl. VIII.

FRUITING ORGAN.

The fruiting organ of this fungus is exceedingly common on all the affected trees and has been collected in Maine, New Hampshire, Vermont, in the Adirondack forests of New York, and in the forests of Toronto, Quebec, and New Brunswick. It is readily distinguished from allied forms by the light red-brown color of the hymenial surface, the regular small round pores, and characters of the hymenial layer shortly to be described.

The form of the pileus varies exceedingly and is almost a distinct one for every host plant. Hartig, in describing what evidently corresponds to this fungus, ascribes the difference in form of the pileus and position on the trees to the different amounts of resin or turpentine which the wood of the different trees contains. *Trametes pini*, according to him, forms brackets around the stump of dead branches in the Pine, the Spruce, and the Larch, while on the Fir the sporophores may appear at any point on the bark. This is true only to a certain extent for the trees of the Northern woods. *Trametes pini* is a very common fungus on nearly all the pines so far seen, and on these trees it always forms very large brackets, which grow, as Hartig says, from old branches. On the Spruce, the Fir, and the Tamarack this does not hold, for on all three of these trees the sporophores form at the ends of old branch stubs and at scattered points on the bark. The resin content of the Spruce is somewhat higher than that of either Tamarack or Fir, and on that account, possibly, the sporophores are more common at the ends of branches. In Pl. XII a number of the forms as they are found on the White and Red Spruces are shown. The bark of these trees consists of corky scales which are constantly being peeled off by newer ones developing beneath. The mycelium of the fungus, after having penetrated through the sapwood of an affected tree, grows rapidly into the younger parts of the bark and ultimately appears as small cushions under several of the bark scales. These cushions are bright red-brown and have a velvety margin composed of thick-walled hyphæ, which rapidly spread out over the adjoining scales, forming a flat sheet (fig. 4). While the growth in a lateral direction is going on, and when the flat sporophore is scarcely one-sixteenth of an inch (about 1.5^{mm}) in width, some of the central hyphæ elongate, leaving small pockets between them which form the pores of the hymenium. The lateral growth may go on for several years, while at the same time a downward growth of the hyphæ which form the walls of the pores brings about an increase in thickness. It ought to be said that this type of sporophore was found only on the under sides of fallen logs or branches. When the sporophores form on a living standing tree they take the form of extended sheets on the lower side of the uppermost branches or form as sessile brackets of varied shape around old stubs of branches or again as sessile brackets at scattered points on the side of the main trunk. Fig. 3 shows a sporophore growing on the under side of a branch. In such a case the mycelium grows out through the bark, forming a long velvety cushion oftentimes several feet in length. This cushion rapidly grows laterally, and on its lower surface the pores arise. The growth of such a sporophore may go on for many years. The under side of the branch shown on Pl. XII, fig. 3 was covered for a distance of 10 feet with the brown sporophore. As the latter increases in width it sooner or later develops a free upper surface where the body of the sporophore projects beyond the curved surface of the branch. The cracks appearing in the wood are due to drying. Fig. 6 shows the sporophore as it occurs on the vertical trunk of a living tree. Here a form results which approaches most closely to *Trametes pini* (Brot.) Fr. The mycelium grows out from between the bark scales, forming a small knob or sometimes several beside or above one another. On the lower side the pores soon appear as shallow pits, which are increased in depth by downward growth of the hyphæ forming their walls. The upper surface of the cushions becomes brown and, because of alternate periods of growth and rest, concentric lines arise which are more or less obscured by the hairiness of the surface. In forms of this kind the directive influence of geotropic forces on the position of the pores is very marked. The pores always extend vertically, and on that account when found on a perfectly horizontal surface their openings are almost round. As one passes on into the oblique portion of the lower surface the openings become more irregular and the lower end portions of the tubes are exposed until they appear as hollow grooves. Where for any reason the position of the trunk or branch upon which a sporophore grows is changed, the direction of the pores changes likewise, and instances of this kind are very common.

On old Spruces ends of broken branches are points where the brown sporophores of this fungus may be found almost without exception. Two cases of this kind are shown on Pl. XII, figs. 5 and 7. The Spruce loses many of its branches during windstorms, far more so than the Fir or Tamarack. The butt end of a broken branch keeps on growing after the death of the outer portion, and in that way large knobs are formed which may in time cover the wound entirely. It is an exceedingly slow process, however, and where, as is frequently the case, the branch breaks off at a distance of a foot or more, as shown on Pl. XII, fig. 5, it rarely if ever heals over.[1] Such branches form the places where the spores of this fungus find a most suitable place for entrance into the trunk. The spore germinates and the mycelium grows down through the dead heartwood of the branch. From there it spreads through the heartwood of the trunk, growing both up and down. The growth in these directions takes place more rapidly than the lateral growth. When the sapwood is reached, the progress is a slow one, owing to the resinous contents. At about this time the sporophore begins to form. The wood of the callus and the living sapwood of the knob become so thoroughly impregnated with turpentine that the mycelium does not grow in them, but grows out through the dead wood of the branch. At the first point where the hyphæ can reach the air without having to go through the collar of sapwood they emerge. Where the dead branch has broken off close to the callus the hyphæ grow out from the stub and form a cushion on it. More frequently, however, the red-brown cushion is formed at the point where the living callus touches the dead wood (Pl. XII, fig. 5). The cushion is at first very small and looks as if covered with velvet. The hyphæ rapidly grow radially and form a sheet which adjusts itself to the shape of the callus and branch. At the edges this sheet projects from the bark and forms an irregular shelf, the top of which after a time becomes zonate and brown-hairy, as in the more strictly bracket-like forms. On many old Spruces there are deep clefts between the various bark scales, and in them sheets of the sporophores form whose folds fill the crevices completely, forming pores on the outer surface of the newer bark and the inner surface of the old scale. Growth takes place rapidly during the latter part of summer and early fall so far as could be noted. The hyphæ at the edge extend the area of the sheet, while those forming the walls of the pores grow vertically downward. Within the pores many hyphæ grow into the holes, so that after a year or two these are completely plugged at the base. There are at present no means of judging how old one of the sporophores described may grow to be. The oldest one found was about four-fifths of an inch (2^{cm}) in thickness.

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[1] There is apparently in the Spruces little of that most efficient natural pruning which takes place in the Pines, where a dead branch breaks off very close to the trunk.

Trametes pini forma *abietis* was found but rarely on the Fir. Its sporophores assume on this tree a different habit from those on the Spruces. On vertical surfaces a distinct sessile pileus is formed, resembling a bracket, rather than a hoof, as do those on the Spruce. The mycelium, after having grown throughout the heartwood, grows into the sapwood, where it flourishes much more vigorously than in the Spruce because of the absence of resin. From the sapwood the hyphæ enter the bark and break through it all over the trunk. At the points where they emerge they form small cushions, light red-brown in color, which are at first the size of a pin head, but rapidly increase in size (Pl. XII, fig. 1). When barely $\frac{2}{25}$ of an inch (2^{mm}) in width, a differentiation into an upper and lower surface takes place. A band of very loosely interwoven hyphæ grows out at right angles to the bark. From the lower side of this band some hyphæ split off and grow downward, adhering closely to the surface of the bark. Other hyphæ also turn down, growing faster at several points than at others, thus giving rise to small pits, which form the beginning of the pores. The pits are very variable in size. When they are still scarcely recognizable the hymenial layer begins to form in them, as evinced by the black cystidia which can be seen projecting from the lower surface of the band first mentioned even before any sign of a ridge is evident to indicate where the next pore is to be. Growth in these directions goes on rapidly. The hyphæ of the original band grow on horizontally, forming a rounded edge of loose hyphæ, which give the hairy appearance to the margin. At intervals, where the growth of the sporophore ceases, some of these loose hyphæ stop growing, and when growth is resumed are left, forming a brush-like projection on the upper surface. These hyphæ give the concentric appearance noted above for the Spruce. The hyphæ on the lower side of the band grow downward to form the pores, and those adhering to the bark grow in the same direction, thus increasing the thickness of the pileus in that direction. A large number of small cushions usually start together on the bark, many of which join as their edges approach one another, forming a series of more or less imbricated sporophores (see Pl. XII, fig. 1). On horizontal surfaces the pileated form is lost, and sheets much like those found in the Spruce are formed. The pores in all the specimens on the Fir are more irregular than those found on the Spruce, but in all other important characters they are identical.

On the White Pine the pileus is sessile and occurs at old knot holes.

On the Tamarack both brackets and sheets are formed. The largest bracket forms found grew on the Tamarack; they often grow singly, and then again together, one above the other. One individual measured 4 inches (10 ^{cm}) in width laterally, 2.8 inches (7 ^{cm}) from front to back, and 2 inches (5 ^{cm}) in thickness at the back along the bark (Pl. XII, fig. 2). The pores in the Tamarack specimens are exceedingly regular, far more so than in those of any of the other sporophores.

The sporophores of *Trametes pini* forma *abietis* grow both on living and fallen trees. They were found on trees which had been cut down four years before, and new ones were constantly appearing. It is this faculty of fruiting on dead trees that must enable this fungus to spread through a forest in a very short time, and accounts for the fact that it does so. After a Spruce has reached a certain age the chances that it will become affected with this parasite are, in the Maine woods, the very greatest. Older trees, i. e., Spruces which have reached a diameter of 10 to 12 inches, are more often subject to attack than younger ones. The fungus enters through any wound, and apparently spreads rapidly. There is no evidence at present to show how rapidly it spreads, nor whether the characteristic form of decay which it induces continues in wood after it has been cut from a tree or not. The present view seems to indicate that it does not grow after the death of the tree.

HYMENIUM.

Hartig[1] has given a very full description and numerous drawings of the hymenial layer of this fungus, and his observations can simply be confirmed. The basidia arise as slender hyphæ, which gradually become much smaller at the apex and form four slender, rather long sterigmata, bearing the spores. These are colorless at first, but turn brown later on, and not infrequently contain an oil globule in the center. The most striking elements of the hymenial layer are the cystidia, called hairs by Hartig. They arise from internal hyphæ, which approach the hymenial layer at an angle. Pushing between the basidia and paraphyses one finds these large, pointed, brown, spine-like bodies, which project for a considerable distance into the pore canal (Pl. IX, figs. 2 and 3). They are thick walled and persist for a long time after the disappearance of the basidia and spores.

As the pores grow older they are filled with a network of hyphæ which grow out from the body of the sporophore, growing over the hymenial layer and completely plugging the hole. The exact period when this takes place was not determined.

POLYPORUS SULFUREUS (Bull.) Fr.

OCCURRENCE.

This fungus, although more frequently found on the hardwood trees, occurs now and then on living Spruces and brings about a brown rot of the wood of trunk and branches. The trees found were attacked after the trunks were 9 inches (23^{cm}.) in diameter. Entrance is effected through wounds and broken branches, much in the same way as the other parasitic fungi which enter above the ground. The mycelium spreads through the trunk of an affected tree, growing up and down, and reaching the highest branches in one direction and the roots in the other. No evidence of a diseased condition is usually visible on the outside, except such as noted for the other diseases.

STRUCTURE OF DISEASED WOOD.

Diseased wood is red-brown in color and can readily be distinguished from wood changed by the other fungi described by the fact that it breaks into slabs or flat pieces, which correspond each to an annual ring of wood (Pl. XIII). The brown rotted wood is hard, very brittle, and breaks into more or less rectangular pieces. When in its final stages, it is exceedingly brittle and can be crushed to a fine powder in a mortar. It is always much firmer than wood destroyed by *Polyporus schweinitzii* and differs from the latter in the character of the cracks or breaks, which are most readily seen on a tangential view.

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[1] Hartig, R. Wichtige Krankheiten der Waldbäume. 50. *pl.* 3. 1874.

The progressive changes which take place in the wood of a Spruce may be noted as follows: The wood at first turns slightly red-brown in irregular patches, as seen when a trunk is split longitudinally. These patches grow larger, spreading from ring to ring and in a longitudinal direction along each ring. Small cracks next appear in these areas, extending part way through the thickness of each ring, both from the side of the spring and of the summer wood. These cracks are very much more visible on the tangential view of an annual ring (Pl. XI, fig. 1). At first but scattered cracks are to be seen extending longitudinally, which, however, soon elongate and pass both diagonally and directly across the direction of the fibers (Pl. XI, fig. 4). At this stage the wood is still hard and has acquired a light-brown color. Immediately about the fissures it is more deeply colored than elsewhere. A microscopic examination shows that there has been great shrinkage in the volume of the cell walls and that the breaks and fissures occur practically throughout the whole mass of the brown wood; though only the larger breaks are visible to the unaided eye. The shrinkage goes on rapidly, and after a time the tension becomes so great that the annual rings separate one from the other. A break usually occurs in a radial direction also, and as a result the free ends of the ring swing outward. Breaks along the lines of the larger medullary rays take place at the same time. This gives rise to long flat slabs of wood, each the width of an annual ring, which hang together loosely at one end and at isolated points on their tangential walls (Pl. XIII). Very badly decayed wood is so thoroughly traversed by larger and smaller breaks that it readily falls to pieces when struck. It must be noted, however, that the nature of the cracks is such that individual pieces of wood are, as it were, mortised into each other end to end, and this no doubt makes the wood as firm as it is.

MINUTE CHANGES IN THE WOOD.

The minute changes which the mycelium of *Polyporus sulfureus* induces in the wood cells are such that they can not well be mistaken. It has been mentioned that the annual rings break into bands which curve inward as the process of drying goes on. A tangential view of several of these bands before they have broken will present an appearance such as is shown on Pl. XI, fig. 4. A large number of fissures have formed both across the wood fibers and parallel with them. The latter are more prominent—the cross fissures never occurring alone, but generally connecting several longitudinal fissures. It will be noted that the breaks are characterized by sharp right angles, and in many places a stepladder arrangement is evident. In the early stages of attack the wood fibers turn red-brown and shrink. As a result, fissures are formed in the walls of the tracheids, which extend diagonally across the wall at an angle of approximately 45 degrees (Pl. XI, fig. 1). The medullary ray cells are at this period still intact, and hold together the more or less brittle wood fibers. The next stage in the decomposition consists in the absorption of the medullary rays. This allows the wood fibers to contract more than up to that time, and as a result breaks occur. These breaks form at first so as to connect adjacent cavities left by the absorption of the medullary rays. The wood fibers tend to curve in one direction or another and break at the weakest point, namely, between two cavities, where the opportunity for curvature is greatest. What determines the direction of curvature of the wood fibers has not yet been explained. In the illustration the curvature is toward the right. This curving has the effect of bringing medullary rays which are in different longitudinal rows approximately into a line. Thus at "*a*" two cavities are shown which are separated by a curved fiber which sooner or later will break, uniting the two. At first two ray cavities are joined, then more, until long longitudinal holes are formed, such as are shown in fig. 4 of Pl. XI. The reason for the sharp angles is now very apparent, likewise why these fissure figures appear only on a tangential view while on the radial view one simply sees the fissures as lines extending at right angles across a ring of wood (Pl. XIII).

The marking of the individual wood cells is a very regular one. The fissures extend through the secondary lamella, and at first sight remind one of those which the mycelium of *Polyporus schweinitzii* induces. The latter are very much steeper, however, and do not occur at such frequent intervals.

The mycelium of *Polyporus sulfureus* is colorless and is present only here and there in the wood cells, a fact to which Hartig calls attention. No spores, such as are so common when this fungus grows in Oak wood, were seen in the Spruce wood, although diligent search was made for them.

FRUITING ORGAN.

The sporophores of *Polyporus sulfureus* are among the commonest and best known of the largest fungi. The sulphur-yellow shelves of this fungus occur widely distributed throughout the United States, and are found in late August and September on many of the Oaks, Walnut, and other broad-leaf trees. A large number of sporophores usually appear together, one above the other, when growing from an upright trunk, or scattered here or there on a prostrate log. They grow on living trees and on the dead trunks also, for several years after the latter have fallen. A marked periodicity in this respect was noted for a particular tree during the past summer. This tree, a large White Spruce, had been blown down some years when first seen. The standing stump was 12 feet (3⅔ meters) in height, and on its south side there developed in August of 1897 a large number of the sporophores. These dried and broke away during the following winter. During the summer of 1898 no sporophores appeared on either the standing stump or the fallen log, and it was not until August, 1899, that a new lot of the brackets appeared, and then in the greatest number. Three large patches broke out on the north and northwest side of the trunk, and the lower side of the fallen log was literally covered with the yellow brackets. No mention of this periodical occurrence of the fruiting portion has been found, and it will be of considerable interest to see what will take place this year. Several other large Spruces in the immediate neighborhood were caused to decay by this fungus, but no sporophores have so far developed on their trunks.

The shape of the pileus varies materially with the position which it happens to occupy. When on upright trunks several sessile sporophores usually occur one above the other, the upper surfaces of the lower ones touching and uniting here and there with the lower surfaces of those above. The individual parts are comparatively thin plates, which have radiating lines and depressions extending outward to the margin. The body of each is soft and fleshy when young and full of a clear yellowish liquid. The upper surface when young is very moist, somewhat hairy, and when bruised turns brown. As the plant grows older it becomes very much harder, and when completely formed is quite hard and brittle. Masses of the young plants have a peculiar fungous odor, which becomes very intense as the parts grow older. The lower surface of the shelf is smooth and even. The pores are formed very early in its development, and almost as soon as they are completed the formation and discharge of spores begin. The sporophores are very short-lived. They begin to appear on the trunk as small rounded knobs, formed by thick-walled hyphæ, which come out from between the bark scales. Their growth is very rapid, even more so than that noted for *Polyporus schweinitzii*. The various small knobs soon flatten into a number of plates, consisting of strands of hyphæ, some of which grow out horizontally, increasing the width of the pileus, while others grow downward to form the pores. When the sporophores develop on the under side of a log they grow out in all directions from a central point, and sometimes forms with a distinct stipe are met with.

Numerous drops of the clear liquid mentioned before were found hanging from the under surface of the shelves on some days.[1] The appearance of the drops does not seem to stand in any relation to the amount of moisture in the air, for they were found alike on very dry and very foggy days. The same sugar, melezitose, that was found in *Polyporus schweinitzii* was obtained from the liquid in quantity. The fungus is attacked when barely mature by insects and small animals, and within a month after the ripening of the spores there is little of it left except the harder upper surface of the shelves and the contracted basal portion. This may account for the fact that the spores ripen and are discharged so very rapidly. Cultures of spores made in water, in sugar water, and on bread showed no signs of germination. These experiments are to be repeated with better cultural facilities.

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[1] Fries notes this fact—Epicrisis, etc. 450.

The spores spread through the air and are carried to all parts of the forest. Wherever any wound or broken branch offers suitable conditions they germinate and induce the rot described.

Polyporus sulfureus was found only on trees growing along the coast of Maine. They were all older trees of the White Spruce. Further search will no doubt show that it attacks the Red Spruce also, and possibly the other conifers. Its large, conspicuous sporophores make its recognition easy, and the fact that they are edible in their early stages ought to lead to their collection and destruction.

POLYPORUS SUBACIDUS Peck.

Poria subacida Peck, Thirty-eighth Report N. Y. State Museum. 92.

OCCURRENCE.

There are a number of fungi which attack standing trees and destroy their wood, of which it is not possible to tell, without continuous observation and experimentation, to what extent they are responsible for the death of trees, and whether they attack perfectly healthy trees. Among these belongs the fungus which for the present will be considered as *Polyporus subacidus* Pk. It is one which is found on decaying logs of coniferous as well as other woods,[1] forming its pores in late summer and winter. It was found once on a living Hemlock, twice on living White Spruce, and once within the trunk of a living White Pine. In many of the spruce forests hundreds of trees, particularly the younger ones, were found dead or dying. Many of these trees were pulled up, and on their roots yellowish masses of mycelium were occasionally found. In one locality some thirty of these young trees, ranging from 2 to 10 inches (5 to 25 cm.) in diameter, had the wood of the trunk decayed by some fungus. The wood appeared yellow, was very wet and spongy, and was easily pulled into shreds. No fruiting organs could be found. Several of the trunks were taken and sawed into pieces a foot (30 cm.) or more in length. These pieces were buried to the depth of a foot (30 cm.) in a sphagnum bank and were examined every week. Other trees were simply broken near the ground and left standing, while in still others wounds were made with an axe to permit the entrance of air, as it was thought that fructification might thus be induced. After two weeks the ends of the pieces buried in sphagnum were covered with a white film of hyphæ, which gradually turned yellow, and after two months began to form shallow pores. The same took place in practically every one of the trees which were overturned or wounded. In all the localities visited where trees, both older and younger, had been overturned, this fungus was found again and again, and associated with it the form of wood decay described below. (Pls. XIV and XV.)

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[1] See Exsiccati, E. & E., N. A. Fungi.

Masses of yellowish mycelium were sometimes found growing out from under the bark scales of the roots of many healthy spruces in a way which seemed to indicate that they were beginning to enter the root itself. Hyphæ from these masses extend into the soil, binding together the particles so that dense clumps are formed, varying from the size of a pea to as large as two fists put together. The growth of the hyphæ in the soil is a very rapid one; they can be grown with ease in moist soil and form the peculiar lumps in a few weeks. Pieces of diseased trunks were buried in soil in a greenhouse in September, and in four months the hyphæ had grown through the soil of the bench in all directions. It is thus very evident that this fungus grows in the ground rapidly and that this is probably one of the ways in which it enters standing trees. This is made more probable by the fact that one finds all of the trees in a certain area affected with this fungus, both younger and older ones. Each probably infected its neighbor much in the way in which *Polyporus schweinitzii* does. The fruiting portion of the fungus has been found on living White Pine, Red and White Spruce, Fir, and Hemlock. A large Hemlock, almost 2 feet (0.6 meter) in diameter (near Houlton, Me.), had been blown over and the trunk had broken some 6 feet (2 meters) from the ground. The wood was very soft and showed numerous black spots surrounded by white areas. The fruiting organs were forming in the chinks and crevices of the trunk, and on the stump. The tree was alive at the time it was seen.

STRUCTURE OF DISEASED WOOD.

The decay which the mycelium of this fungus induces is not to be confused with that caused by any other fungus. Spruce wood when very much decayed is moist, almost wet at times, and can be compressed much like a sponge, when quantities of water will drip from the mass. Larger and smaller cavities of very irregular shapes, lined with a tough felt of hyphæ, yellow on the inner side, are found throughout the wood. Such a cavity is shown in part at the bottom of Pl. XIV, fig. 2. The cavities are scattered throughout the wood in most trees and are generally partially filled with a pale straw-colored liquid. The wood itself differs markedly in different trees. This difference appears to be due somewhat to the rapidity with which the solution of the fibers takes place. As a rule, the wood in the early stages of the attack has numerous black spots scattered throughout its mass (Pl. XIV, fig. 1). These black spots are surrounded by a white circle before long, and somewhat later disappear entirely, leaving very much larger white spots. The wood around the spots is now straw-yellow in color and begins to look somewhat frayed, as if groups of wood fibers were separating readily from the rest. A tendency for the different annual rings to separate now becomes very marked (Pl. XIV, fig. 1, at the right), and a log of spruce wood at this stage can be split into concentric rings by mere pounding. Gradually the number of white spots increases. In one form of decay the white spots are confined almost entirely to the summer wood. The newly formed spots are also in the summer wood, and before very long all the summer wood of every ring, including also some of the adjacent spring wood of that ring, has turned white. This stage of decomposition is shown very well in Pl. XIV, fig. 2, a longitudinal section of a spruce log, and in Pl. XV, fig. 1, a cross section of the same log. It will be noted that the change to the white masses nowhere passes from the summer wood of one ring to the spring wood of the adjoining ring. There is evidently some agent, presumably of a chemical nature, which confines the solvent action of the fungus mycelium to the summer wood and prevents it from attacking the spring wood. It may be recalled here that where a similar change takes place in the spruce wood, induced by the mycelium of *Trametes pini* forma *abietis* (Pl. X, fig. 2) both summer and spring wood were changed. This localized action of the dissolving agent takes place with such regularity and in so many different ways, depending upon the kind of fungus attacking the wood, that it suggests the presence of specifically distinct dissolving agents, enzymes, perchance, for each fungus.

In the second form of decay the appearance of the white spots is limited to the summer wood in the same way as above described. The white spots do not increase in number so rapidly and consequently do not form the white bands spoken of. Changes take place within the wood cells of the spring wood, which give to them a very light and porous nature. A cubic inch (16.4^{cc}) of such wood completely decayed weighs but 1.3 grams (sound spruce wood weighs 5.52 grams).

The mycelium of the fungus spreads through the individual tracheids after entering the tree, and collects in spots here and there. Solution of the wood cells begins around these centers, which at this time appear dark brown or black. They are the black spots referred to above. The change which takes place around these centers consists in a solution of the hadromal and the other lignin constituents of the cell walls, leaving the pure cellulose fibers free from one another. These constitute the white spots and also the white bands spoken of. The various steps leading to the complete separation of the cellulose fibers are exactly those which have been described for a similar process caused by the hyphæ of *Trametes pini* forma *abietis*.

A very different change is going on at the same time in the spring wood, and gradually spreads from this to the summer wood. This change may be likened to the one which Hartig has described as taking place in pine wood attacked by *Polyporus borealis*.[1] The hyphæ of the fungus develop in the wood cells with great rapidity, filling them completely. Numerous hyphæ pass through the walls in all directions, making large irregular holes many times the diameter of the hyphæ which pass through them. The secondary walls of the wood cells are gradually dissolved; a faint granular appearance of the walls is seen at first, and little by little the walls become thinner. At last only the primary lamella is left, and in the bordered pits the torus (Pl. XI, fig. 3). The whole wall finally disappears, leaving simply that part of the wall belonging to two or three cells, namely, the portion having a triangular cross section. This solution of the walls goes on simultaneously throughout large areas. The medullary rays disappear completely, long before the wood cells are entirely gone. The spaces left by the dissolved cells are rapidly filled with hyphæ and these hold portions of the cell walls not yet destroyed in place, and give consistency to the mass, which thus retains the shape of the wood before the attack. The whole mass can be compressed by slight pressure and will not return to its original size. This accounts for the extremely light weight of wood thus decayed. In Pl. XI, fig. 2, a radial view of wood in an advanced stage of decay is shown. The straight black lines indicate groups of wood vessels, two or more; the hyphæ between

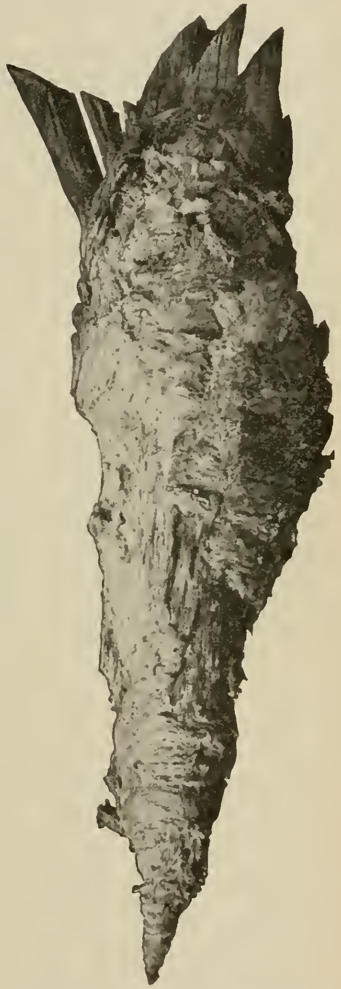

FIG. 3.—Base of spruce branch, showing its resistance to the attack of the mycelium of *Polyporus subacidus* Pk.

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[1] Hartig, R. Zersetzungserscheinungen des Holzes, etc.

them have dissolved out the missing fibers and now fill the spaces. Plate XI, fig. 3, represents a cross section of similarly attacked spruce wood, showing several wood fibers of the spring wood at one side and the gradual dissolution of adjoining ones, leaving only the more resistant portions which lie free in the masses of hyphæ. These remaining parts stain with phloroglucin and hydrochloric acid, showing that they are still lignified walls. Heartwood and sapwood of the spruce are destroyed with equal rapidity. All parts become spongy, with the exception of the resinous basal pieces of the branches, which resist the attack of the fungus even after the whole trunk has been destroyed. This resistance of the basal pieces of the branches is quite a common feature in diseased trees attacked by several other fungi, notably *Polyporus schweinitzii*, but nowhere is it more striking than in this instance. Text figure 3 shows such a branch piece as it appeared immediately after pulling it from a dead standing tree.

FRUITING ORGAN.

After the mycelium has invaded the sapwood it grows out over the bark, forming yellow felts. This takes place in the early part of the summer, generally about July. A few weeks later the small pores begin to form. Certain hyphæ of the sheet turn at right angles to it and grow out at this angle, forming shallow pores. These are almost round and are separated by very thin dissepiments. Fig. 2, Pl. XV, is from a photograph of a spruce log, about the middle of September, almost natural size. As the season progresses the fungus dies and splits up into smaller areas and some of the tubes become inclined. No pores occur at the edge of the sheet, thus leaving a fringe of sterile hyphæ. This distinguishes this fungus from many allied forms. The hymenial layer and the pores are generally straw yellow, sometimes even more decidedly yellow, the color deepening toward the latter part of the fall. The pores do not form until December or January, and as a completely fruited fungus was collected but once, its description will be deferred until more material has been seen.

The fruiting organ frequently develops in cracks and breaks formed when a diseased tree is blown over. Fructification was induced in many instances, as described above, by allowing the air and moisture to have access to completely decayed wood.

When *Polyporus subacidus* grows in Northern forests on dead coniferous wood as a saprophyte, its habit and action differs somewhat from that described above. Inoculation experiments were made during the summer to test how rapidly this fungus destroys sound wood. Diseased wood from both dead and living trees was placed in holes bored in healthy spruces, and the latter were labeled so as to be readily identified in later years. The amount of destruction which this fungus does in the spruce forests is very large, but careful experiments will have to be made to determine its relation to trees weakened by other causes, also its progress through the soil from tree to tree.

REMEDIES.

This fungus may be accounted most destructive to dead timber, and any remedies spoken of for *Polyporus pinicola* apply here. Dead trees should be utilized before the chance for infection becomes too great. No practical remedies can be suggested at present to prevent its spread through the soil.

OTHER DISEASES.

Besides the diseases described in the foregoing there are a number of others of which not enough was seen to enable a full description to be given.

POLYPORUS VAPORARIUS (PERS.) FR.

This is frequent on Spruces and Firs, and induces a brown rot of the sapwood. The fungus occurs widely spread over the United States and Canada on all coniferous woods. Its fruiting body is very variable, and there are probably many fungi included under this name which do not belong there. From observations made in the Maine woods it seems that this fungus attacks dead much more than living trees, destroying them for timber very rapidly. A fuller description of it will be given at a later date.

POLYPORUS ANNOSUS FR.

This fungus is a parasite of European trees much feared by the foresters of the Continent. Diligent search was made for it, but fully formed fruiting bodies were not found. A single Spruce seen on the top of Mount Kineo, Moosehead Lake, had its roots covered with firm leathery sheets, such as *Polyporus annosus* sometimes forms on the roots of the Southern Pines. Unfortunately there were no means at hand to cut down the tree, so that an inspection of its trunk was impossible. Other diseased trees of Spruce and of the Fir were seen north of the Rangeley Lakes. One of these was overturned, having grown in a damp locality. Its roots were covered with the yellowish leathery felts which extended into the surrounding soil. The trunk of this tree was completely rotted in the center, the decay going up the trunk for 25 feet (almost 8 meters). At this point the wood was brown, showed some white areas, and smelled strongly of prussic acid. The stumps of many other Spruces were examined for evidences of this fungus. Some Spruces were found which had small holes in the summer wood of many annual rings. The wood when cut longitudinally showed many of these holes, which differed from those formed by *Trametes pini*.

They occurred chiefly in the summer wood, and were filled with a red-brown powder. There is no white lining as in the wood attacked by *Trametes pini.* Black spots appear here and there in the wood, and when they disappear the holes take their place. The holes increase in size and number, and in the last stages of decomposition the wood has become a shredded mass of yellow-brown fibers, which feel much like straw. It is completely honeycombed in every direction. The annual rings of wood separate from one another, forming thin plates perforated by thousands of small holes. The transformation of this fibrous material takes place from the root up into the trunk for from 3 to 20 feet (1 to 6 meters). In some trees the innermost rings of wood are affected. As the wood becomes more and more rotted a hole is formed which gradually increases in diameter, eventually sometimes becoming so large that the weakened trunk is blown over by the wind. On other trees one or the other side of the trunk may be affected. Two or more separate holes may be formed which join near the base of the tree.

A more lengthy description of the changes in the wood just described is not deemed necessary, in view of the fact that the active agent which brings about the changes is as yet not fully determined. If it proves to be *Polyporus annosus* Fr. it would seem that the injury done in the Eastern forests by this fungus is not very large, which may be considered a fortunate circumstance, as this fungus is one naturally to be dreaded by the forester, as it is combated only with the greatest difficulty and expense.

AGARICUS MELLEUS VAHL.

Many trees were found in which the well-known rhizomorph strands of this fungus grew under the bark. The summer of 1899 was exceedingly dry, and on that account the development of Agaricineæ of all kinds was a very meager one. On the various excursions made through the Maine forests but one tree was found on which the yellow fruiting organ of this fungus was developing. The manner in which this fungus grows on the roots of the trees and brings about their death has been so fully described by Hartig and others that it seems hardly necessary to describe it here. The fungus grows within the living roots and cambium of a tree and speedily brings about a disturbance in its absorbing organs which results in ultimate death. The wood is rarely if ever affected to any extent, so that lumbermen use the diseased trees for lumbering purposes, making no distinction between them and live trees as long as the wood is entirely sound. Diseased trees should be cut at once when recognized.

CONCLUSION.

The conditions in the New England forests are very favorable to the growth and development of timber-destroying fungi, conditions which are made still more favorable by an ever increasing supply of dead wood. Radical changes will be necessary in the present lumbering methods in certain localities before any betterment can be hoped for. During the summer of 1899 the wasteful cutting of timber was noticed in particular in the region north of the Moosehead Lake, where the old system of measuring logs by the top scale is still in vogue. The lumberman cuts the logs on the stumpage plan, and in his endeavor to obtain as high a scale as possible he cuts the tree high up on the trunk and low in the top, leaving almost half the top in the woods. This is not only wasteful lumbering, but offers an excellent opportunity for the development of several of the fungi described in the foregoing pages. From the dead trunks and limbs their spores spread to standing trees which might otherwise remain sound. The same is true for the insects, as recently pointed out by Hopkins.[1]

In the foregoing it has been pointed out that as trees grow older they become more liable to insect and fungus attack. An old tree has many vulnerable points, such as old branches and wounds made by animals or by hail, where insects or fungi may gain entrance to begin their work of destruction.

As a tree grows older the chances that it will be attacked become greater. This point ought to be taken into consideration in the harvesting of a timber crop. In certain sections of the Maine forests, particularly in the Rangeley Lake region, the trees have reached an age where it appears that the rate of annual accretion, and consequently the annual increase in value, is very small, while the danger of infection is increasing every year. It is recommended that such trees be cut immediately where practicable, as they are practically ripe and probably at their point of greatest value. This may not always be possible, owing to practical difficulties in reaching water courses, etc., but the principle should be established that it will prove more profitable in the long run to cut trees after they have reached a certain age, to prevent depreciation due to the attack of fungi or insects. Future investigation will have to determine what the exact age is at which it will be most profitable to do this cutting.

It has also been pointed out that there are several fungi which attack trees after they have been killed by insects or other agents. This is of great practical significance, for it may often be possible to harvest such dead trees before the fungus in question has had time to begin its work.

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[1] Hopkins. A. D. Preliminary Report on the Insect Enemies of the Forests of the Northwest. Bul. No. 21, Div. of Entomology, U. S. Dept. Agr. 1899.

In the Maine forests great areas of forest lands were killed by bark beetles some years ago. If the dead trees had been cut shortly after their death, the timber might have been utilized, and it would have been as valuable as that from live trees, for the beetles do not mine in the heartwood. This was not done, however, and before long the whole forest of dead trees was rendered worthless by several fungi, notably *Polyporus pinicola* and *Polyporus subacidus*. What is true of larger areas holds for individual trees in the forest, and also in those sections where strong winds blow over many trees. Such an area, technically known as a windfall, offers opportunities for the action of destructive fungi, and the same recommendations just made for areas where trees are destroyed by insects hold good. A dead tree is as valuable as a live tree, provided its wood is sound, and it ought to be cut immediately. There is some prejudice among lumber bosses that such trees are of no account; nothing can be further from the truth, and this fact ought to be insisted on by those in charge of cutting operations.

The trees, now in the forest, which are diseased are beyond help, and it is at present neither practicable nor economical to practice the methods in use by the European foresters, which consist in the prompt removal and destruction of the diseased trees. The time will come when this may prove profitable in the regenerated forests, but for the present the most hopeful method of combating fungi is by conservative lumbering. Men who are acquainted with the manner in which insects and fungi work and who can direct the cutting operations ought to be employed.

It may not be out of place here to refer to the growing sentiment in favor of restricted cutting, which was very much in evidence in the localities visited. Much agitation is still going on decrying the lumberman as the greatest enemy of the forest; but with the growing realization that it is possible to utilize the timber of the forest and still leave a forest which will yield timber from year to year, this feeling is gradually lessening. The lumberman has not been slow in realizing that restricted cutting will be more economical in the long run than the indiscriminate destruction of the past years. It is gratifying to note that two of the largest lumber owners of western Maine are employing trained foresters, under whose directions the cutting operations are carried on.[1] These men will not only be able to make operations more profitable, but can also aid in gathering information which may go to solve many of the problems still to be unraveled in connection with the enemies of forest trees.

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[1] See also Graves, Henry S. The Practice of Forestry by Private Owners. Yearbook, Dept. of Agr. **1899**: 415. 1900.

EXPLANATION OF PLATES.

PLATE I.

Fig. 1. Sporophores of *Polyporus schweinitzii* Fr.

Fig. 2. A piece of the bark of Red Spruce with sporophores of *Polyporus volvatus* Peck growing from holes formed by a boring beetle, a species of *Dendroctonus*.

PLATE II.

Cross section ($\times\frac{3}{4}$) of the trunk of a living young Balsam Fir (*Abies balsamea* (L.) Mill.) at a point 4 feet (1.2 meter) from the ground. Decay, caused by *Polyporus schweinitzii* Fr., has shrunk the wood, producing a number of cracks and giving it a rough appearance. It is so nonresistant that the saw tore the fibers instead of cutting them. The large crack at the top, extending through the sapwood, was formed when the tree was cut down. A small sporophore of the fungus grew at the base of this tree.

PLATE III.

Radial view ($\times\frac{1}{2}$) of a log of White Spruce (*Picea canadensis* (L.) B. S. P.), showing an early stage of decay induced by the mycelium of *Polyporus pinicola* (Swartz) Fr. The fine parallel lines indicate the annual rings of wood. Here and there white spots with darker centers are seen; likewise long white lines parallel to the course of the wood fibers, and others near the center of the figure, which extend in an irregular manner across the direction of the fibers.

PLATE IV.

Radial view ($\times\frac{1}{2}$) of a log of White Spruce (*Picea canadensis* B. S. P.), showing an advanced stage of decay induced by mycelium of *Polyporus pinicola* (Swartz) Fr. The wood has cracked throughout. The white masses are sheets of mycelium. At the right of the figure two sporophores are shown—one just beginning to develop, the other about 1 year old. The sapwood has been partially destroyed by boring larvæ, whose tunnels are filled with sawdust.

PLATE V.

Three sporophores ($\times\frac{1}{2}$) of *Polyporus pinicola* (Swartz) Fr. The uppermost one is a young one. The one on the right is growing on a stump, and its lower surface is much eaten by insects. The one on the left is a very old sporophore, in which the ridged upper surface is very marked.

PLATE VI.

Fig. 1. Radial view of a piece of wood (natural size) of the Red Spruce (*Picea rubens* Sargent), showing an early stage of the decay induced by the mycelium of *Trametes pini* (Brot.) Fr. forma *abietis* Karsten. The white spots indicate where the wood has been changed so as to leave cellulose fibers. Small black lines are visible here and there.

Fig. 2. Radial view of Red Spruce log (natural size), showing advanced stage of the same decay. The number of white spots has increased. The decay rarely goes beyond this stage.

PLATE VII.

Radial view of a log of Balsam Fir (*Abies balsamea* (L.) Mill.), showing advanced stage of decay due to *Trametes pini* (Brot.) Fr. forma *abietis* Karsten.

PLATE VIII.

FIG. 1. Piece ($\times\frac{3}{4}$) of wood of tamarack or larch (*Larix laricina*), showing early stage of the decay caused by *Trametes pini* (Brot.) Fr. forma *abietis* Karst. Note how the annual rings separate at one end.

FIG. 2. Piece ($\times\frac{3}{4}$) of tamarack wood, showing an advanced stage of the same decay. The piece is composed of very little sound wood; the larger portion is cellulose.

PLATE IX.

FIG. 1. Radial view of two spruce tracheids, showing the manner in which cracks appear in the walls when such wood is destroyed by *Polyporus schweinitzii* Fr.

FIG. 2. A pore from the sporophore of *Trametes pini* (Brot.) Fr. forma *abietis* Karst., growing on *Abies balsamea*, showing numerous cystidia projecting from the hymenial layer.

FIG. 3. Enlarged view of a portion of the hymenial layer shown in fig. 2, showing cystidia with thick walls and several basidia with spores.

FIG. 4. View ($\times\frac{1}{2}$) of the lower surface of an old pileus of *Polyporus pinicola* (Swartz) Fr., of which a portion has died. This is shaded dark. Hyphæ from the living parts are forming a new layer, which is slowly covering the dead parts. The pores are indicated by the dots.

FIG. 5. Young sporophore (natural size) of *Polyporus pinicola*, cut in the middle to show arrangement of pores and top.

FIG. 6. Resupinate form (natural size) of pileus of the same fungus.

FIG. 7. Older pileus (natural size) of the same fungus, sectioned through the middle.

FIG. 8. Diagrammatic representation of a section through the pores of *Polyporus pinicola*. They are continuous from year to year. A firmer layer of hyphæ, incrusted with crystals of oxalate of lime, forms a line of demarcation between successive growth increments.

FIG. 9. Cross section of wood elements from summer wood of Spruce (*Picea rubens* Sarg.) attacked by *Trametes pini* forma *abietis*, showing how the fibers are gradually changed until only cellulose is left; "w," unchanged wood fibers; "b," the outermost lamellæ (unshaded) now consist only of cellulose; "c," more advanced stage; "e," the middle lamella is being converted into cellulose, and is finally absorbed, leaving only portions "p" free among the white cellulose fibers.

FIG. 10. Radial view of tracheids from wood of Spruce (*Picea canadensis* (Mill.) B. S. P.) attacked by *Trametes pini* forma *abietis*, in the region of a hole fringed by a black line. (See Pl. VI, fig. 2.) The tracheids are filled successively with hyphæ, which are incrusted with a brown material so as to completely plug the tracheid.

FIG. 11. Tracheid from wood of Spruce (*Picea canadensis* (Mill.) B. S. P.) during early stage of attack by *Trametes pini* forma *abietis*, showing hyphæ.

FIG. 12. Hymenial layer of *Polyporus pinicola* (Swartz) Fr.

FIG. 13. Radial view of white area from wood of Balsam fir (*Abies balsamea* (L.) Mill.) attacked by *Trametes pini* (Brot) Fr. forma *abietis* Karst., showing how the hyphæ gradually recede from a center, forming plugs in every wood element. The plugs are colored almost black by a brown product of decomposition.

PLATE X.

FIG. 1. Cross section of Spruce wood partially destroyed by mycelium of *Polyporus pinicola*. Large cavities and breaks which are filled with fine hyphæ are being formed in the wood. The summer wood is indicated by the parallel shading, the hyphæ by dots; "c," a small fissure enlarged in text figure 2. The lines at the left $=0.5^{\mathrm{mm}}$.

FIG. 2. Cross section of a piece of Spruce wood, showing early stage of destruction by *Trametes pini* forma *abietis*. Parallel lines of holes filled with cellulose fibers, here indicated by dots, appear in the wood. The black lines bounding the cavities simply indicate the limit of change of cellulose, for in reality there is no such sharp line of demarcation. The short line at the right equals about $\frac{1}{25}$ of an inch (1^{mm}).

FIG. 3. Later stage of the same form of decay. The wood is now simply a network of narrow wood lamellæ separating larger and smaller holes. In these lamellæ black lines are shown, which represent plugs of brown hyphæ incrusted with decomposition products. (See Pl. IX, figs. 10 and 13.) Cellulose fibers and mycelium fill some of the cavities. The short line at the base equals about $\frac{1}{25}$ of an inch (1^{mm}).

FIG. 4. Longisection of wood (Spruce), showing effects of destruction by hyphæ of *Polyporus pinicola*.

FIG. 5. Cross section of several wood cells, showing changes which take place in wood such as shown in fig. 4.

PLATE XI.

FIG. 1. Tangential view of Spruce wood destroyed by mycelium of *Polyporus sulfureus* (Bull) Fr.: "a" wood elements which have been curved, bringing two medullary rays into line; "e" part where a break occurred, uniting two medullary rays.

FIG. 2. Radial view of wood in last stage of decay, induced by mycelium of *Polyporus subacidus* Pk. The straight black lines represent one or more wood elements held in place by the hyphæ which are wound all around them. Remnants of medullary rays are to be seen here and there.

FIG. 3. Several cells from such a piece as is shown in fig. 2 (also Pl. XIV, fig. 3). Normal wood cells of the spring wood are shown at the left, and going toward the right various stages in the solution of the cell walls.

FIG. 4. Tangential view of a piece of Spruce wood destroyed by mycelium of *Polyporus sulfureus*, showing characteristic breaks in the wood, formed by the uniting of many medullary rays by cross breaks. (See fig. 1 of this plate.) The short line at the left is equal to 1^{mm}.

PLATE XII.

Various forms of sporophores of *Trametes pini* forma *abietis*.

FIG. 1. On Balsam Fir.

FIG. 2. On Tamarack.

FIG. 3. On horizontal branch of Spruce.

FIG. 4. On bark of trunk of Spruce.

FIG. 5. At base of dead branch of Spruce.

FIG. 6. Semipileate form on Spruce.

FIG. 7. At base of dead branch of Spruce.

PLATE XIII.

Radial view of a block of White Spruce (*Picea canadensis* (Mill.) B. S. P.) partly destroyed by mycelium of *Polyporus sulfureus*. The darker spots at one side show where the wood turns brown and ultimately cracks. The manner in which the annual rings separate is indicated near the top of the figure.

PLATE XIV.

FIG. 1. Radial view of White Spruce (*Picea canadensis*), showing early stage of destruction by *Polyporus subacidus* Pk.

FIG. 2. Radial view of White Spruce log showing destruction of wood by mycelium of *Polyporus subacidus* Pk. The white lines show where the wood has been so

changed as to leave cellulose fibers. Near the bottom of the figure note a cavity lined with mycelium.

Fig. 3. Radial view of White Spruce wood decayed still further by the same fungus. The wood is soft and flaky and is being changed to cellulose here and there.

PLATE XV.

Fig. 1. End view of a Spruce log similar to the one shown on Plate XIV, fig. 1, showing how the summer wood of every annual ring has been changed, leaving cellulose fibers.

Fig. 2. View (about natural size) of the resupinate sporophore of *Polyporus subacidus* Pk. on Spruce log, showing how it creeps over the bark.

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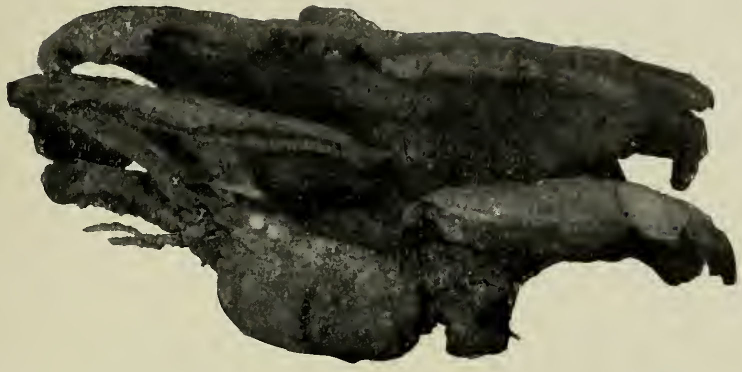


FIG. 1.—SPOROPHORES OF POLYPORUS SCHWEINITZII FR.

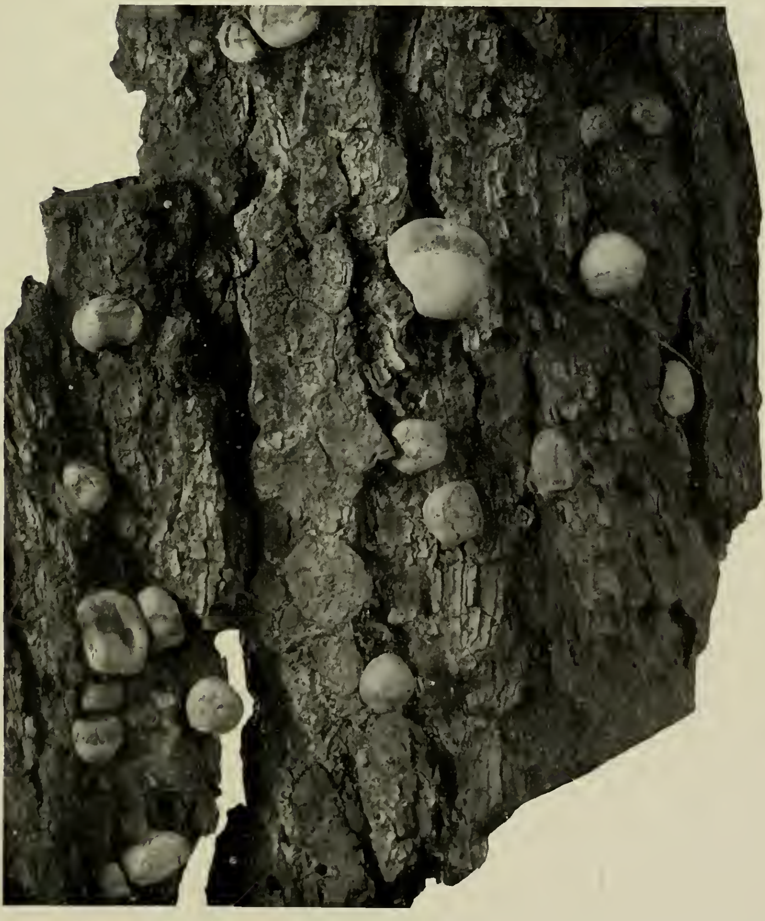
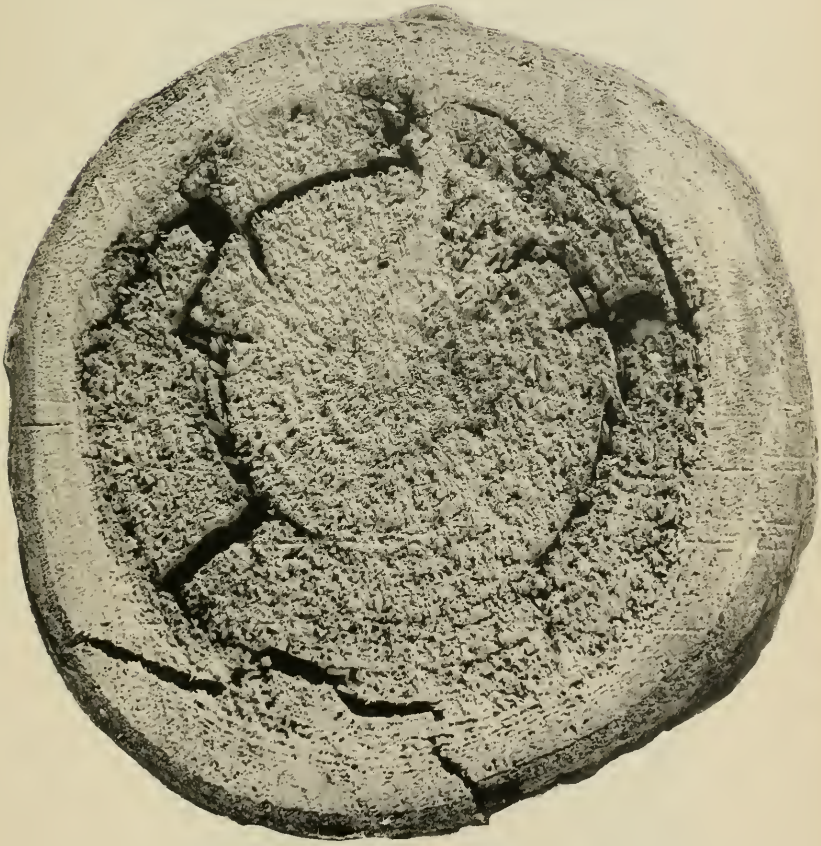
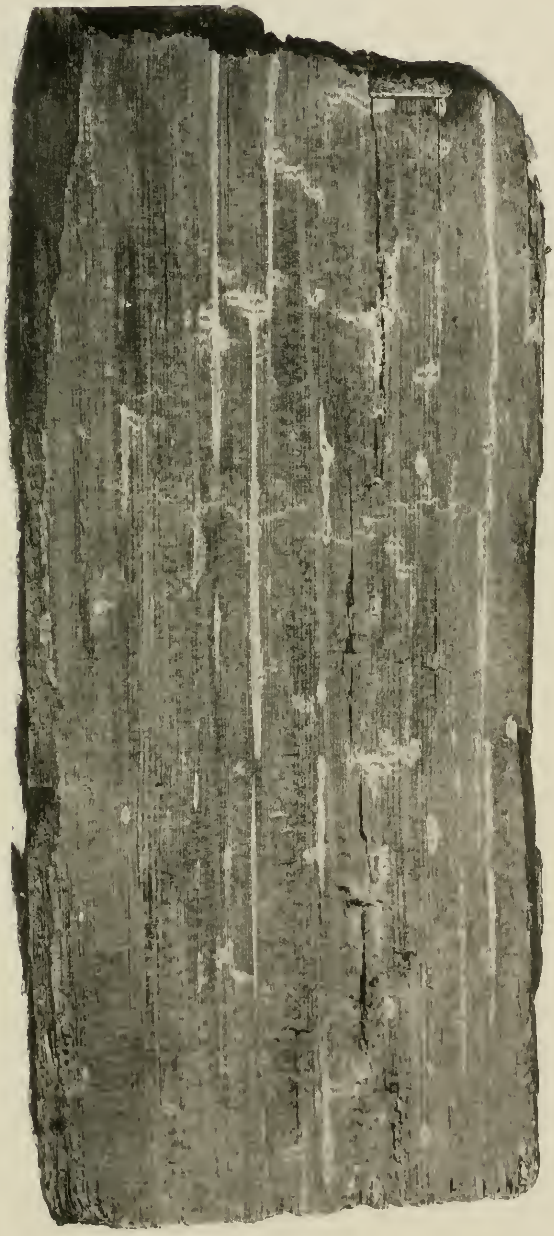


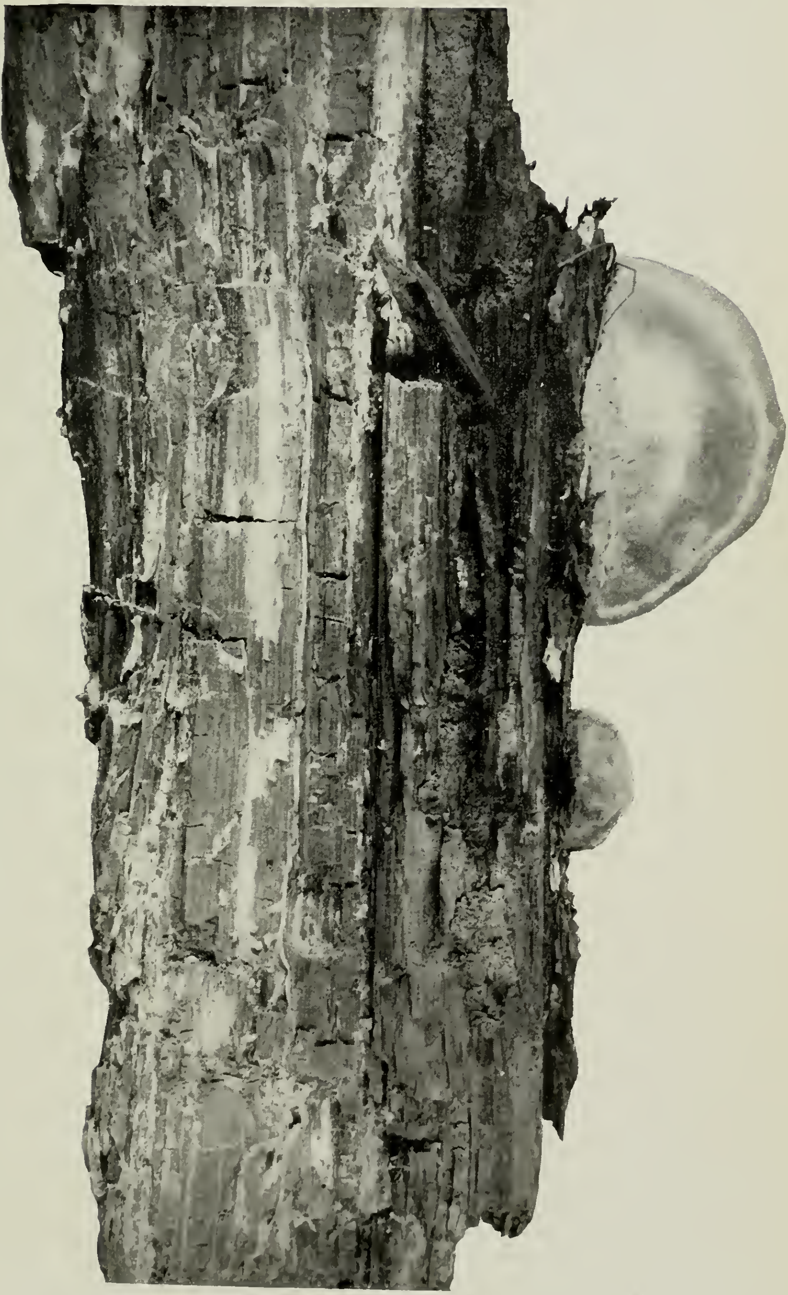
FIG. 2.—POLYPORUS VOLVATUS PECK, GROWING FROM HOLES MADE IN THE BARK BY DENDROCTONUS SP.



Log of Balsam Fir showing decay caused by Polyporus schweinitzii Fr.

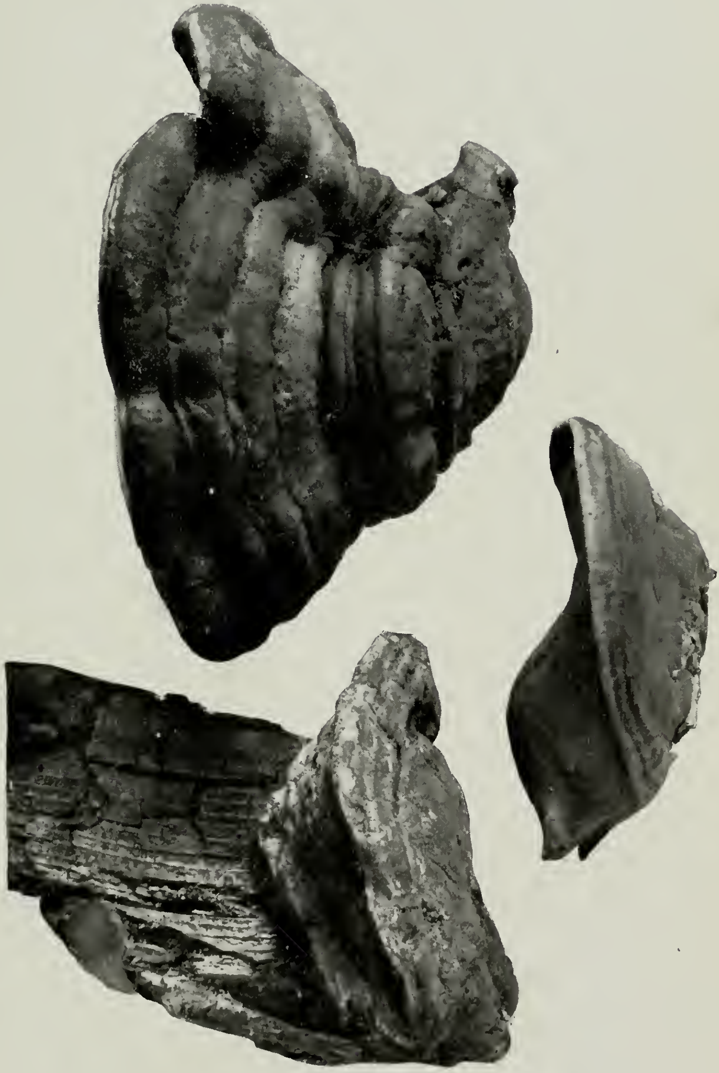


LOG OF WHITE SPRUCE SHOWING EARLY STAGE OF DECAY CAUSED BY POLYPORUS PINICOLA (SWARTZ) FR.



LOG OF WHITE SPRUCE SHOWING ADVANCED STAGE OF DECAY CAUSED BY POLYPORUS PINICOLA (SWARTZ) FR.

PLATE V.



SPOROPHORES OF POLYPORUS PINICOLA (SWARTZ) FR.

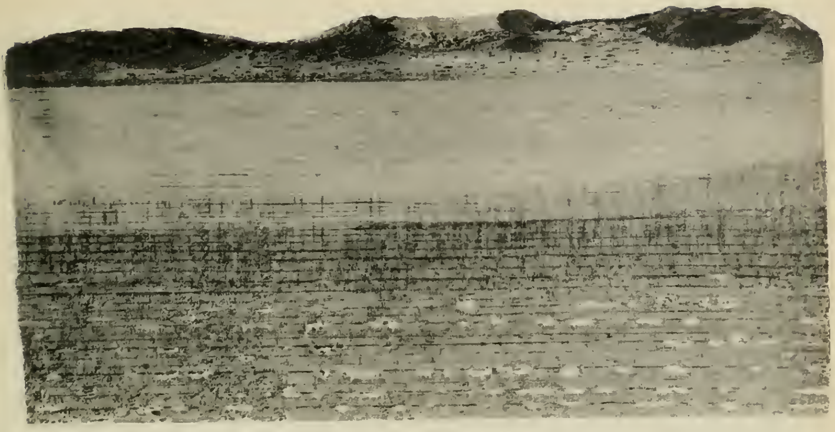


FIG. 1. RED SPRUCE: EARLY STAGE OF THE DECAY CAUSED BY TRAMETES PINI FORMA ABIETIS

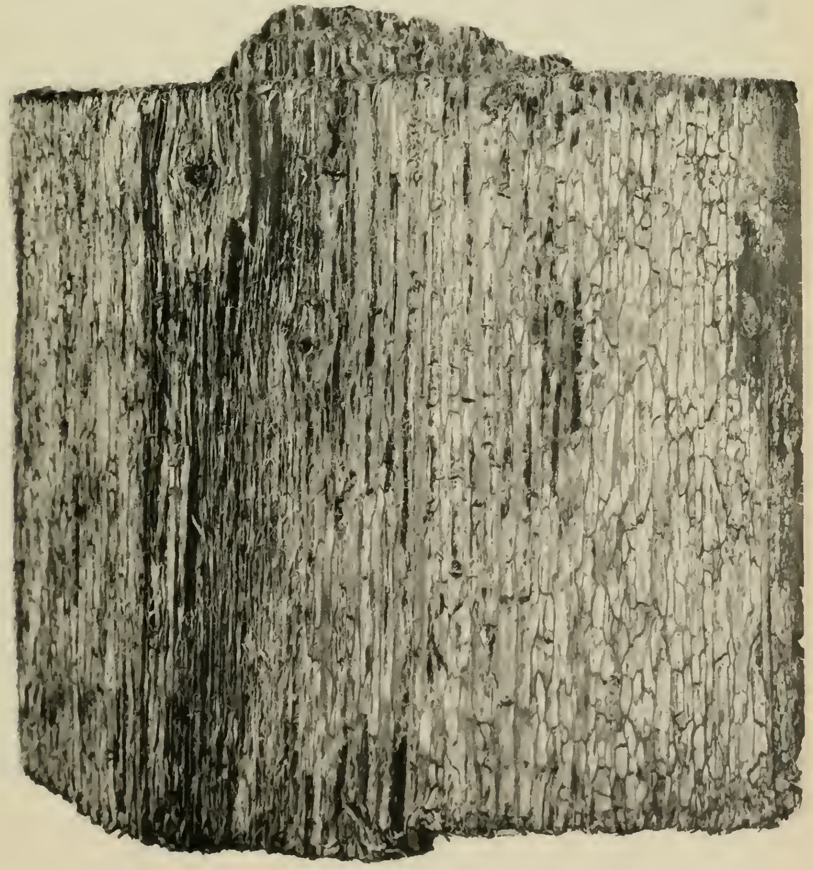
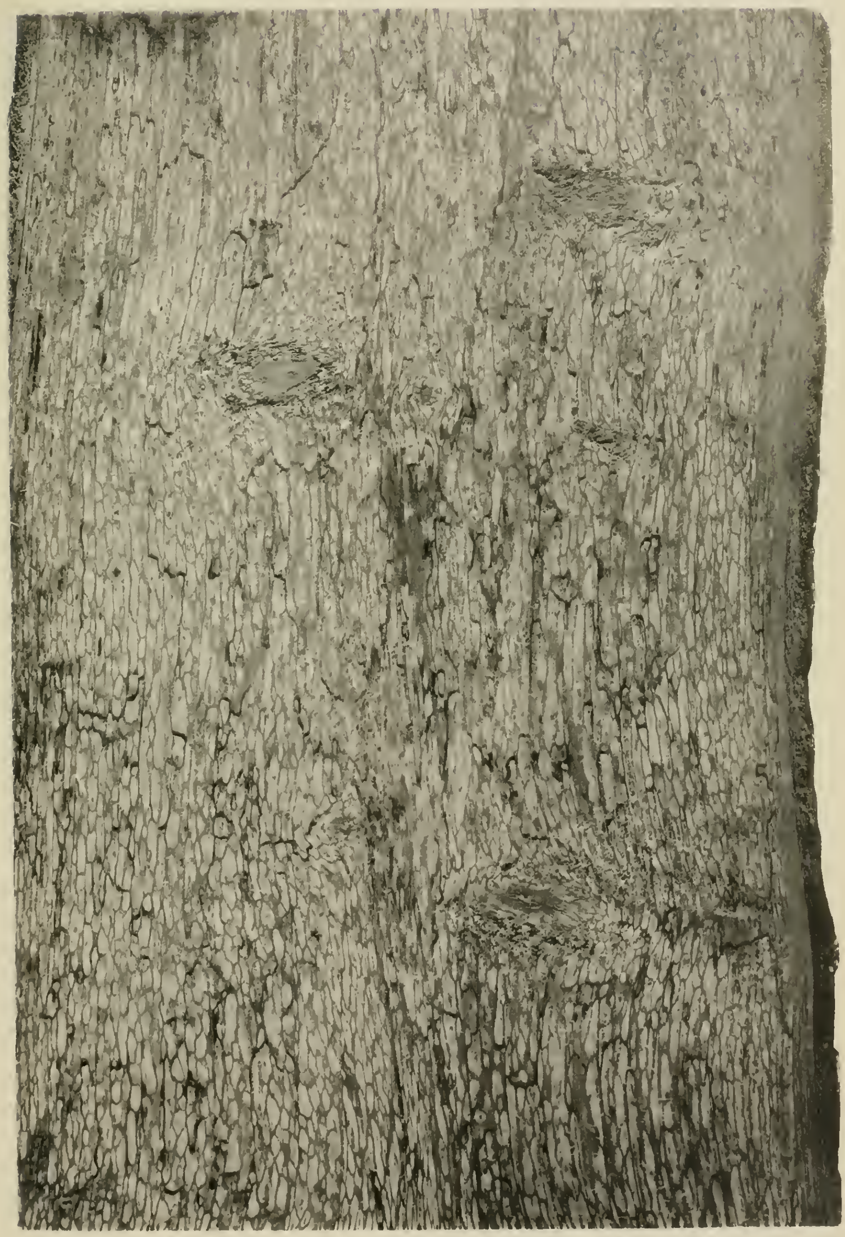


FIG 2. RED SPRUCE: ADVANCED STAGE OF THE DECAY CAUSED BY TRAMETES PINI FORMA ABIETIS.



Log of Balsam Fir showing decay caused by Trametes pini forma abietis.

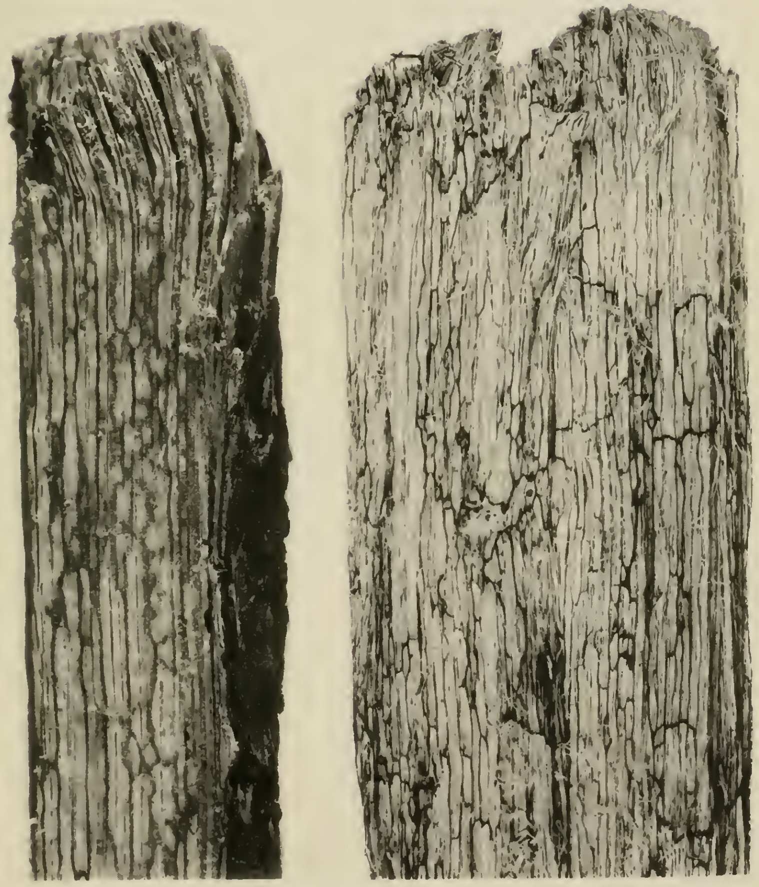
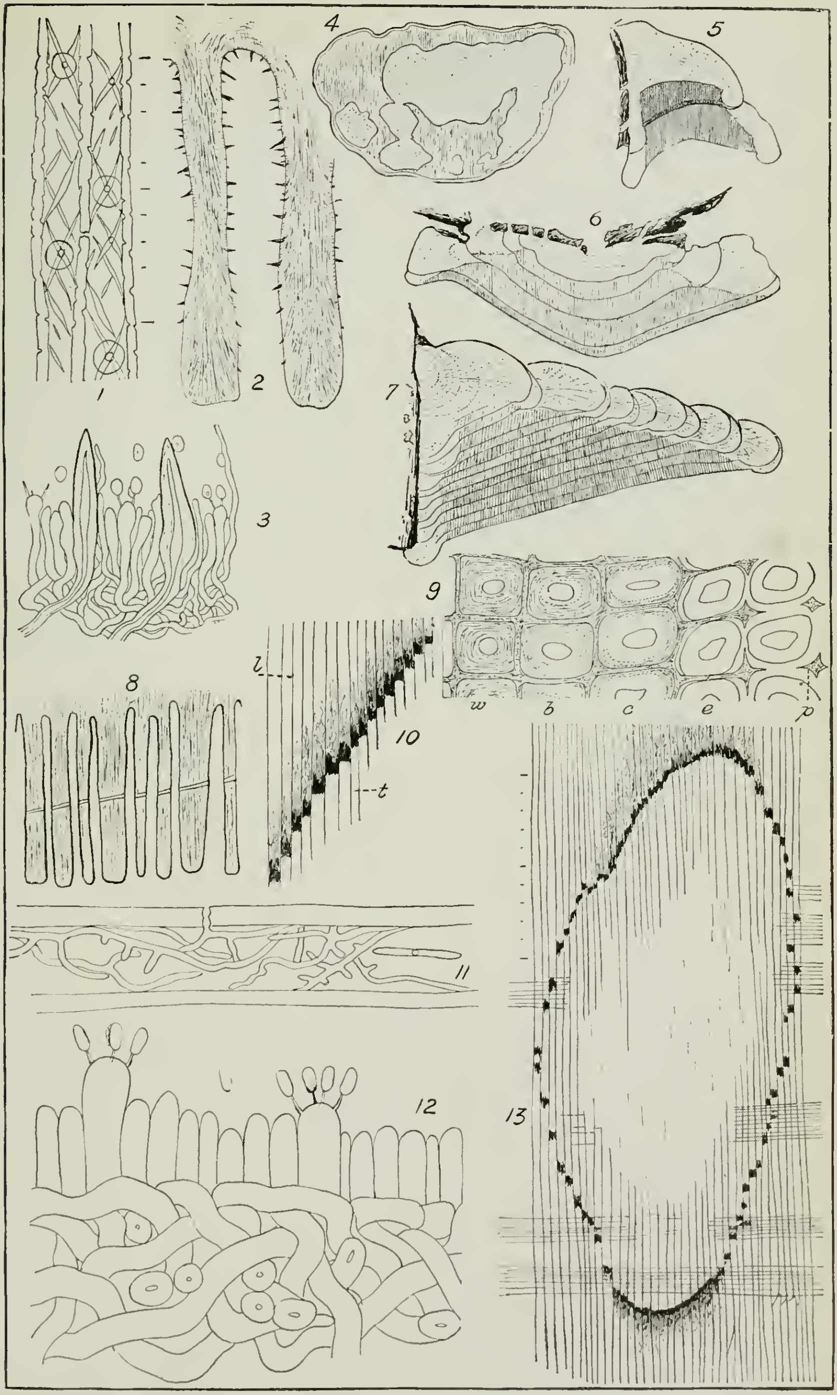


FIG. 1. FIG. 2.

FIG. 1 EARLY STAGE AND FIG. 2 LATE STAGE OF DECAY OF LARCH CAUSED BY TRAMETES PINI FORMA ABIETIS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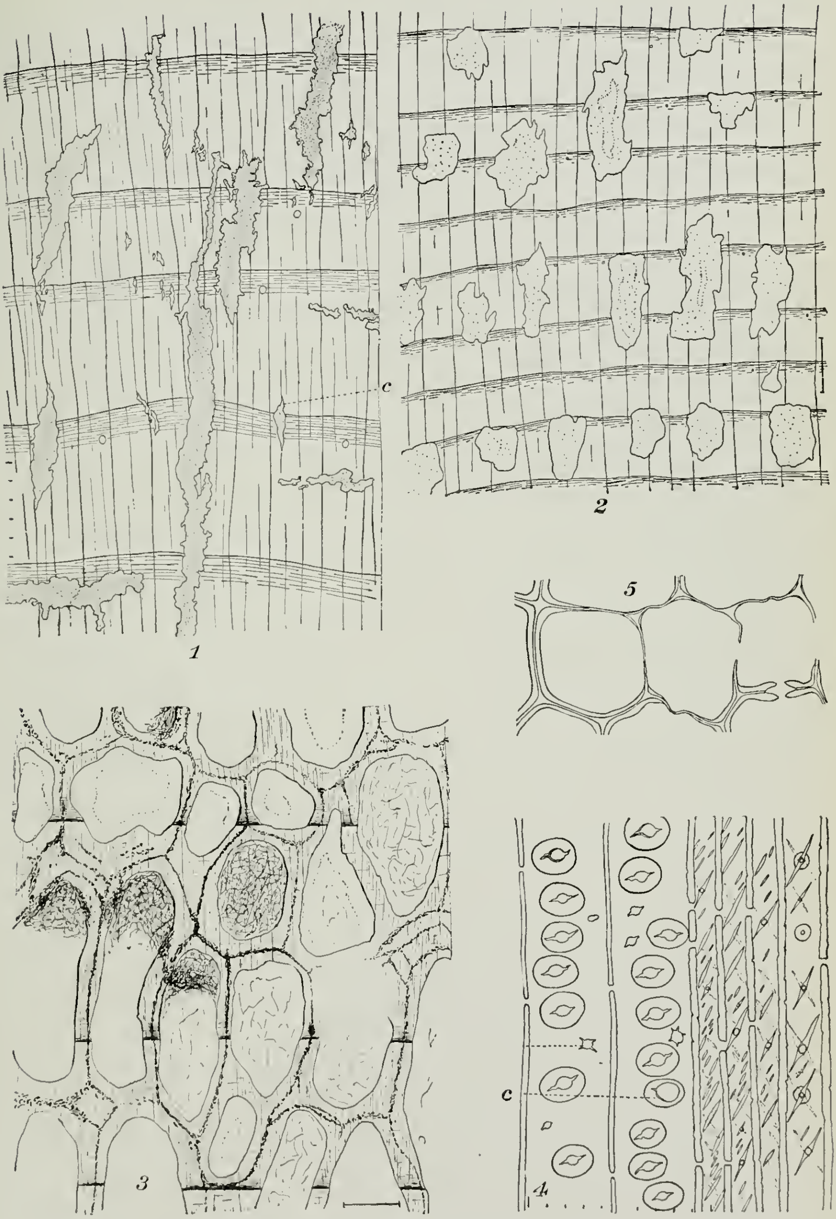
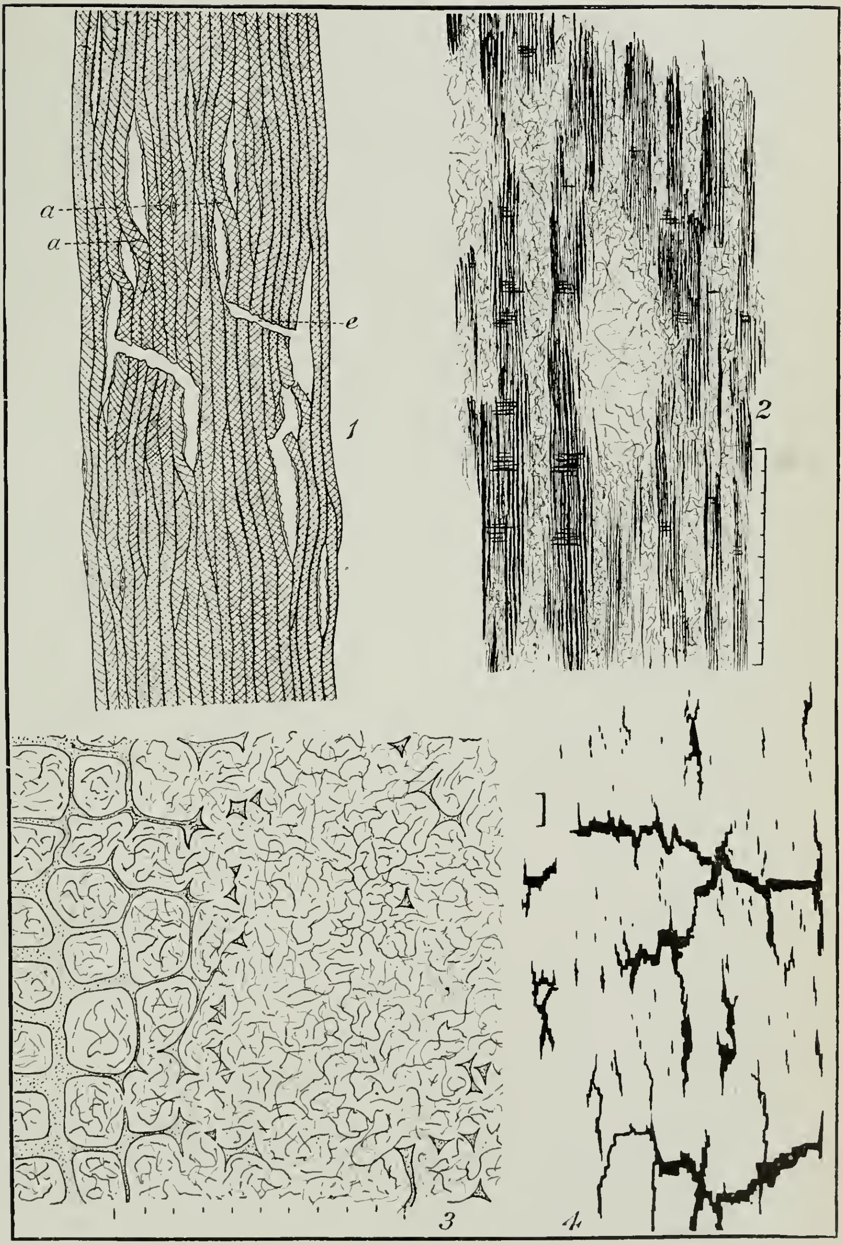
POLYPORUS SUBACIDUS PK., POLYPORUS PINICOLA (SWARTZ) FR., AND TRAMETES PINI (BROT.) FR. FORMA ABIETIS KARST.

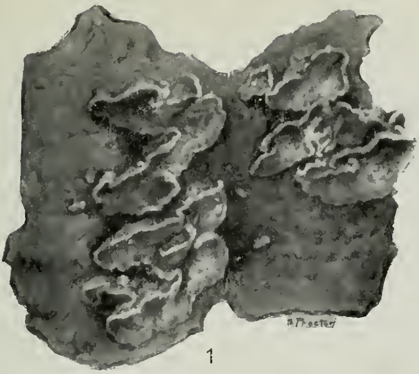
PLATE X.



Work of Polyporus pinicola (Swartz) Fr. and Trametes pini (Brot.) Fr. forma abietis Karst



STAGES OF DECAY INDUCED IN SPRUCE BY POLYPORUS SUBACIDUS PK. AND POLYPORUS SULFUREUS (BULL.) FR.



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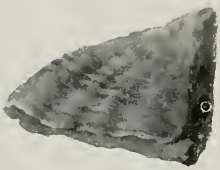

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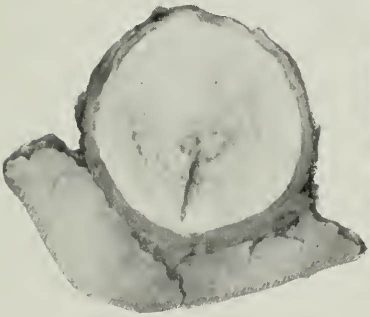

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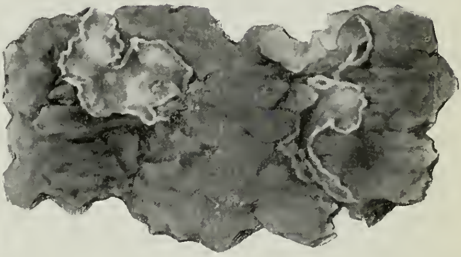

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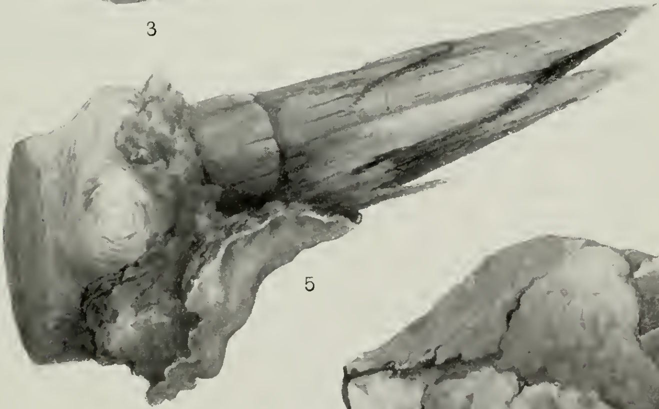

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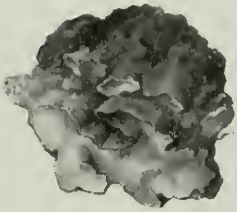

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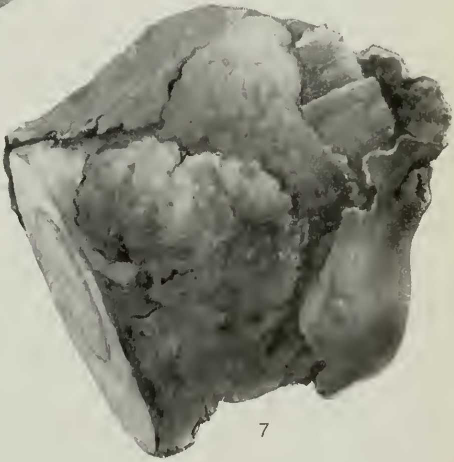

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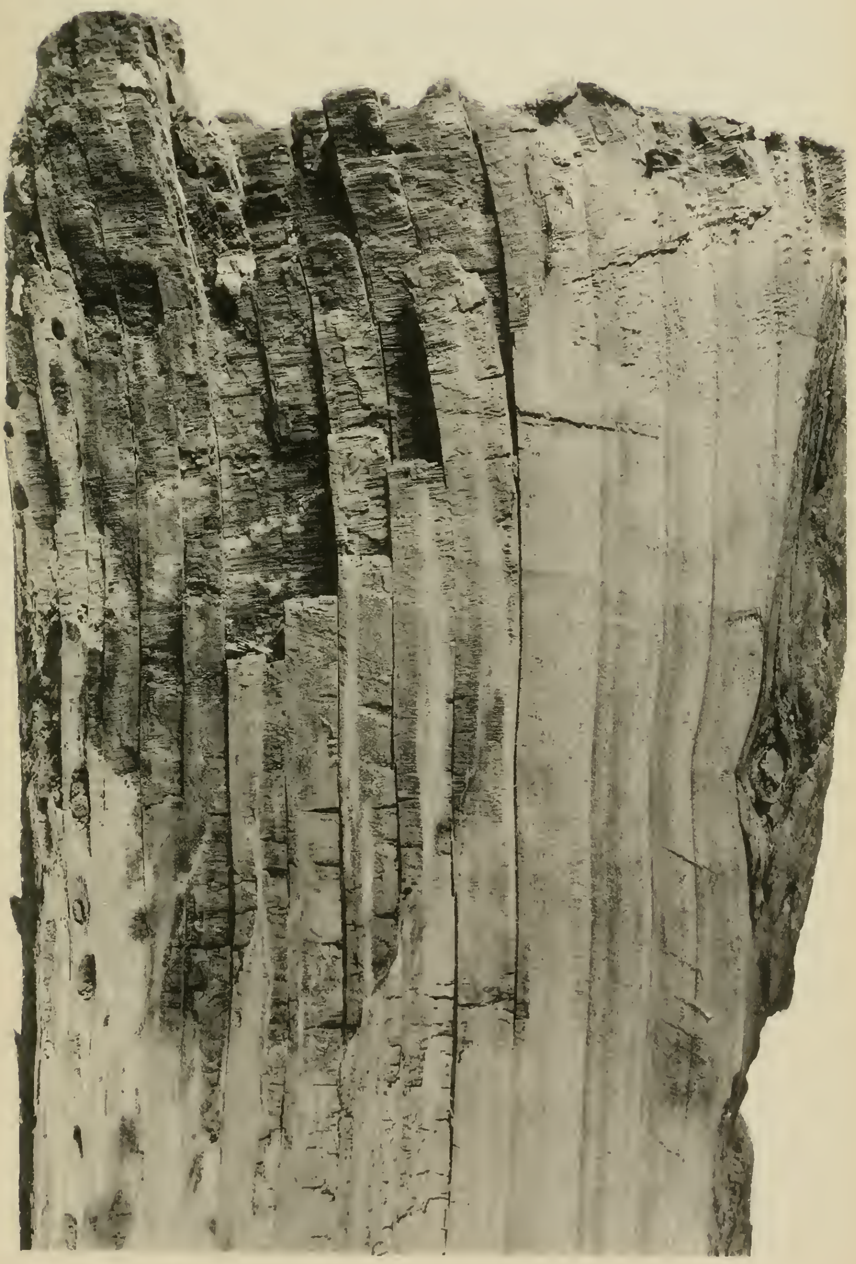
VARIOUS FORMS OF SPOROPHORES OF TRAMETES PINI (BROT.) FR. FORMA ABIETIS KARST.



BLOCK OF WHITE SPRUCE WOOD SHOWING INJURY CAUSED BY POLYPORUS SULFUREUS.

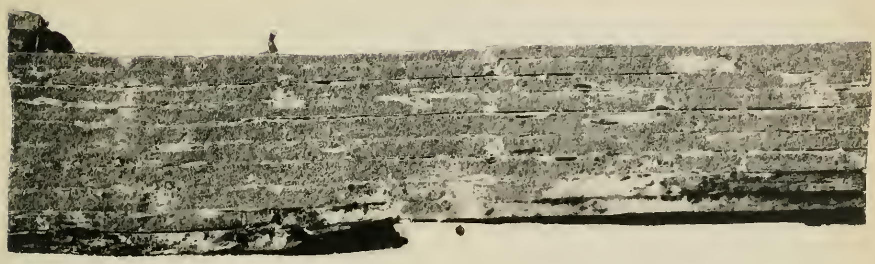


FIG. 1.

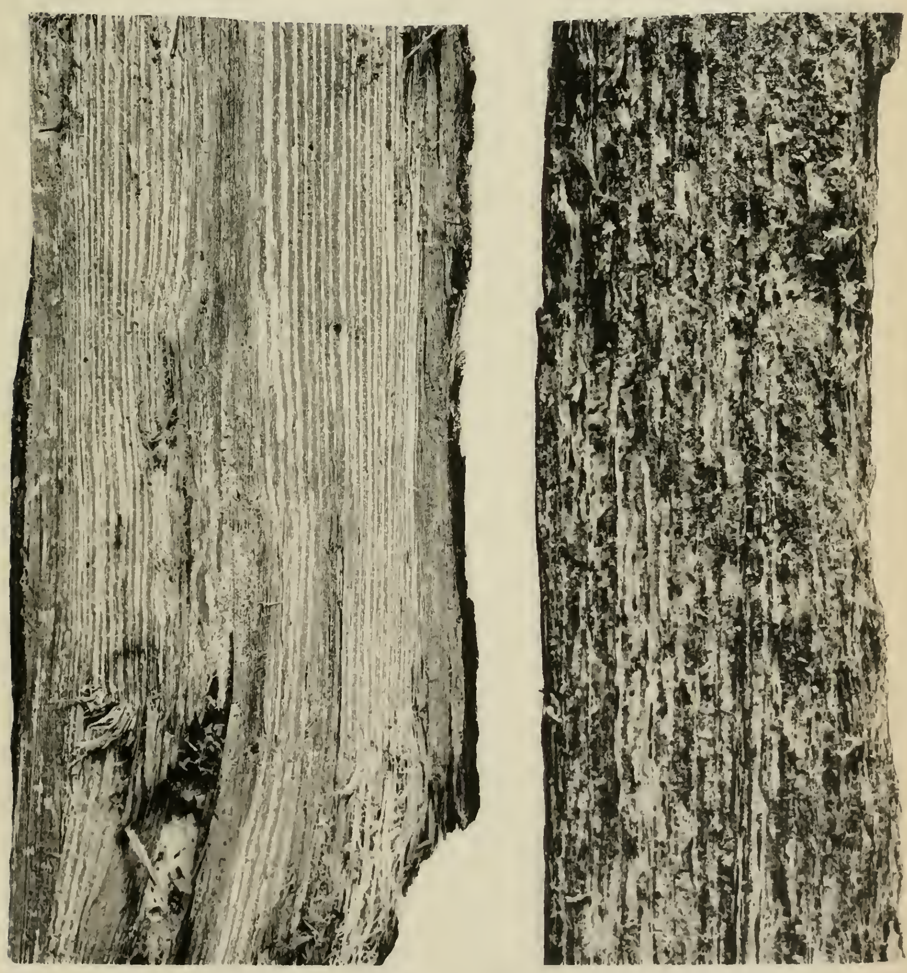


FIG. 2. FIG. 3.

FIG. 1 EARLY STAGE AND FIGS. 2 AND 3 SUCCESSIVELY LATER STAGES OF THE DECAY CAUSED IN WHITE SPRUCE BY POLYPORUS SUBACIDUS PECK.

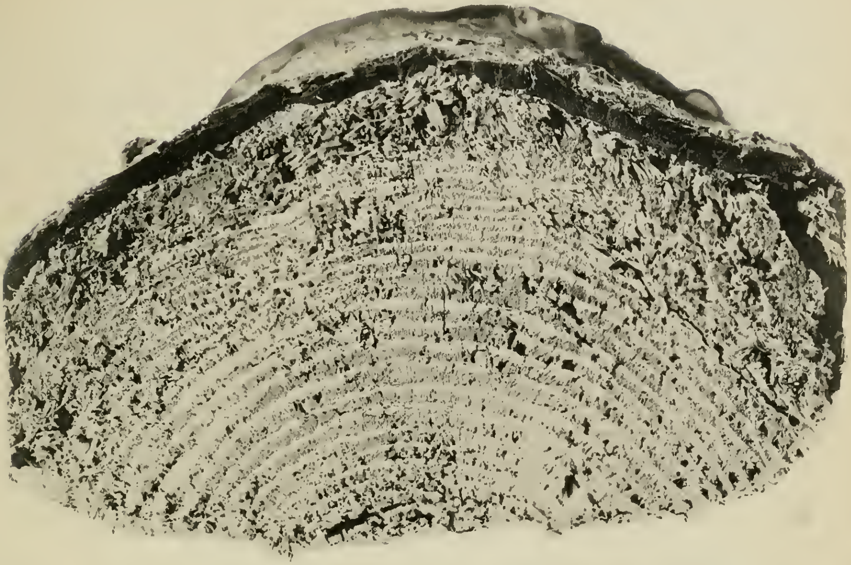


FIG. 1. CROSS SECTION OF LOG OF SPRUCE SHOWING DECAY CAUSED BY POLYPORUS SUBACIDUS PECK.

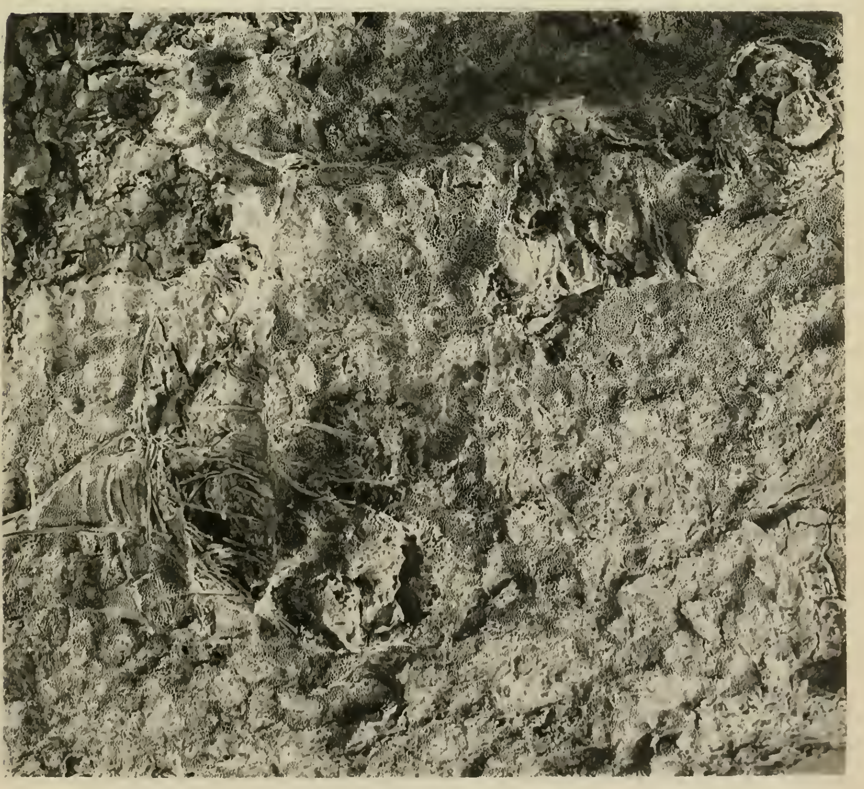


FIG. 2. RESUPINATE FORM OF SPOROPHORE OF POLYPORUS SUBACIDUS PECK ON SPRUCE LOG.

BULLETIN No. 26. V. P. P. 79.

U. S. DEPARTMENT OF AGRICULTURE,
DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY.
ALBERT F. WOODS, CHIEF.

WAKKER'S HYACINTH GERM,

Pseudomonas hyacinthi (Wakker).

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ERWIN F. SMITH,
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1901.

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U. S. DEPARTMENT OF AGRICULTURE,
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1901.

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Division of Vegetable Physiology and Pathology,
Washington, D. C., October 6, 1900.

Sir: I respectfully transmit herewith and recommend for publication a report by Dr. Erwin F. Smith, of this Division, on a bacterial disease of hyacinths commonly known as "the yellow disease" or "Wakker's disease."

The fact that large numbers of hyacinth bulbs are forced each year in the United States makes it desirable that their diseases be understood. The information gained regarding the biology of the organism will also be of great value to those investigating the bacterial diseases of plants.

The report confirms earlier work done in the Netherlands and adds much new and important information respecting the nature of the parasite. The latter belongs to a group of bacteria, hitherto but little studied, several members of which (also studied by Dr. Smith) cause diseases widely prevalent in the United States.

The report while primarily for pathologists and bacteriologists will also be of interest to florists and any others who wish to detect this disease and to avoid its introduction into the United States.

Respectfully,

Albert F. Woods,
Chief of Division.

Hon. James Wilson,
Secretary of Agriculture.

PREFACE.

This paper was prepared for publication in August, 1897, at which time I had secured characteristic infections and had worked out many of the cultural and other characters given in the following pages. The fact that I had not again produced the disease with germs isolated from my first series of infected plants, the further fact that I could not satisfactorily explain the meager growth of the parasite in the host plant, and on steamed potato and the other culture media which I had used, and, finally, a shadow of doubt concerning the accuracy of two or three other observations, induced me to withhold the paper and repeat the experiments. In the time which has intervened I have gone over nearly or quite all of the experiments detailed in the original paper, without, however, discovering any serious errors. During this time reinfections have been secured, the reason for the feeble parasitism has been discovered, and a number of other interesting facts have been brought to light, so that the long delay of publication has not been without its compensations.

Throughout this study numerous comparisons have been made with two other yellow bacteria, *Pseudomonas campestris* and *Ps. phaseoli*, and occasional mention has been made of them in this paper, both being plant parasites. Occasional comparisons have also been made with other bacteria, especially with *Ps. Stewarti.* The leading cultural characters of the hyacinth organism are mentioned in the synopsis at the end of this paper, but it has been decided to relegate an account of the numerous experiments on which these conclusions rest to a second bulletin, which is now ready for publication and in which they will be discussed in connection with the cultural peculiarities of the other yellow species of Pseudomonas here mentioned.

It is too much to hope that this bulletin is entirely free from mistakes. Nevertheless great pains have been taken to make it trustworthy, all of the experiments having been performed in duplicate, and nearly all of them having been repeated several times on different occasions to eliminate unsuspected sources of error.

Some brief statements respecting the morphology and physiology of this organism, as determined by the writer, were made at the Detroit meeting of The American Association for the Advancement of Science in August, 1897, and were published in the Proceedings of the Association for that year (Vol. XLVI, 1897, Salem, June, 1898).

ERWIN F. SMITH.

CONTENTS.

Page.

Historical 9

Source of material 10

Inoculations of 1897 10

  Series 1 (hyacinths) 10

  Series 2 (onion) 18

Natural infection of a daughter bulb 18

Inoculations of 1898 19

  Series 3 (hyacinths) 19

  Series 4 (onions) 20

  Series 5 (hyacinths) 20

  Series 6 (Roman hyacinths) 23

  Series 7 (hyacinths) 23

  Series 8 (hyacinths) 25

  Series 9 (hyacinths) 26

  Series 10 (hyacinths) 26

  Series 11 (cabbage) 27

  Series 12 (amaryllis) 28

  Series 13 (hyacinths) 28

  Series 14 (hyacinths) 29

  Series 15 (onions) 30

  Series 16 (hyacinths) 30

  Series 17 (hyacinths) 31

  Series 18 (plunge experiment) 32

Inoculations of 1899 33

  Series 19 (hyacinths) 33

Remarks on pathogenesis 33

Morphology of the parasite 36

  Size and shape 36

  Motility 37

  Zoogloeæ 38

  Spore formation 39

  Involution forms 39

Behavior toward stains 40

Synopsis of characters 40

Remarks on relationship 42

Explanation of illustrations 44

ILLUSTRATIONS

PLATE.

Page.

PLATE I. *Pseudomonas hyacinthi* (Wakker) Erw. Sm 46

TEXT FIGURES.

FIG. 1. Diseased scale of hyacinth 22

2. Inoculated leaf of hyacinth 23

3. Culture of *Pseudomonas hyacinthi* on slant 30 per cent cane-sugar agar, showing "shagreen" surface 38

4. Slightly magnified diagrammatic views of slime of *Ps. hyacinthi* on sweet potato, showing "shagreen" surface 38

5. Typical behavior of *Ps. hyacinthi* in fermentation tubes containing peptone water, or peptonized beef bouillon, with addition of various sugars and other carbohydrates 41

6. *Ps. hyacinthi* growing in strongly alkaline (0) gelatin with 10 per cent cane sugar. No liquefaction. The surface curves are due to the very gradual drying out of the gelatin 42

WAKKER'S HYACINTH GERM,

Pseudomonas hyacinthi (Wakker).

HISTORICAL.

Dr. J. H. Wakker published five papers on the hyacinth germ between 1883 and 1888. His studies attracted wide attention because he was one of the earliest investigators in the field of plant bacteriology in a time of general skepticism and uncertainty, and also because of the great care with which he seemed to have worked out his results. Since the conclusion of Dr. Wakker's studies, which were begun in 1880, no bacteriologist or plant pathologist seems to have given any personal attention to the disease. Several pathologists have written about it or referred to it,[1] but nothing of any value has been added, and some of the comments have served only to throw doubt on the original inquiry.

In reading Dr. Wakker's papers for the purpose of making an abstract, I was at once struck with the need of a reinvestigation of the subject. This seemed necessary for two reasons: (1) Methods of isolation were not then as well understood as at present, and most of Wakker's successful infections seem to have been direct ones; (2) the germ is so imperfectly described that, excluding the test of pathogenesis, the identification of any particular organism as *Bacterium hyacinthi* Wakker would be altogether impossible. No disparagement of Dr. Wakker's beautiful studies is here intended. At the same time nothing perhaps better serves to illustrate the important advances which have been made in the technique of bacteriology than a perusal of the best early papers.

---

[1] De Bary: Vorlesungen über Bacterien, Leipzig, 1885, p. 137; also 3. Auflage, Leipzig, 1900, p. 173.

Sorauer: Handbuch der Pflanzenkrankh., 2. Auflage, 2. Theil, Berlin, 1886, p. 99.

Kramer: Die Bakteriologie in ihren Beziehungen zur Landwirtschaft, etc. Erster Theil, Wien, 1890, p. 145.

Comes: Crittogamia Agraria, Napoli, 1891, p. 510.

Ludwig: Lehrbuch der niederen Kryptogamen. Stuttgart, 1892, p. 96.

Tubeuf: Pflanzenkr. durch kryptogame Parasiten verursacht, Berlin, 1895, p. 556.

Prillieux: Maladies des Plantes agricoles et des Arbres fruitiers et forestiers causées par des parasites végétaux, Paris, 1895, Tome I, p. 22.

Frank: Die Krankheiten der Pflanzen, 2. Auflage, 2. Band, Breslau, 1896, p. 23.

Migula; System der Baketrien, 1. Bd., Jena, 1897, p. 320.

Hartig: Lehrbuch der Pflanzenkrankheiten, 3. Auflage, Berlin, 1900, p. 209.

It is not unlikely that the additions which I shall make will also be insufficient, exclusive of the pathogenic test, to differentiate this germ ten or twenty years hence, but they will at least help toward definitely settling the group to which it belongs. Readers who wish merely a summary of Dr. Wakker's conclusions will find it in my critical review already cited, and those who wish to read the original papers will find the necessary references in the same paper.[1] Inasmuch as that review is very full and readily accessible, I may be excused from going over the ground again in this place.

SOURCE OF MATERIAL.

The hyacinth bulbs from which the germ that I have studied was isolated were said to be in the first stages of the yellow disease, and were sent to me in October, 1896, by Messrs. Van Meerbeck & Co., growers of bulbs at Hillegom, near Haarlem, Netherlands. The bulbs were sound externally. They had been "visited,"[2] and some of the vascular bundles of the inner scales were yellow, broken down, gummy, and full of bacteria. Penicillium was also present in places. No difficulty was experienced in isolating a yellow micro-organism from the broken down bundles of one of these bulbs, and subsequently the same germ was isolated from another bulb of the same lot. By planting a third bulb the disease was also obtained the following year in a daughter bulb. I have now cultivated this organism over four years in hundreds of cultures on a great variety of media, and have also obtained very satisfactory infections—infections so exactly like those described by Dr. Wakker that there can be no doubt either as to the nature of the organism with which I have worked or as to the substantial accuracy of Dr. Wakker's conclusions respecting its pathogenic properties.

INOCULATIONS OF 1897.

SERIES 1 (HYACINTHS).

The first set of inoculations was made February 16, 1897, from a pure beef-broth culture. Eight vigorous hyacinths were inoculated. They were all of one variety, a robust, single-flowered, deep-blue sort (name unknown). The plants were just coming into blossom and were the picture of health, six of the eight bulbs being large and well-stocked with food, and the other two smaller daughter bulbs. Part of the inoculations were by means of ordinary needle punctures and the rest by means of a hypodermic syringe, the results being the same, except that the symptoms appeared sooner when a large number of germs were inserted. All of the inoculations were made in the middle or terminal parts of healthy leaves, with one exception, in which case the germs were inserted into the upper part of a flower shaft before the buds opened.

---

[1] The Bacterial Diseases of Plants: A Critical Review of the Present State of our Knowledge, Parts III and IV, *The American Naturalist*, October and November, 1896, pp. 797, 912.

[2] Removal of the top of the bulb with a sharp knife for purposes of inspection is called "visiting." This is done after the bulbs are dug.

Much to my surprise, the progress of the disease was very slow, exactly as described by Dr. Wakker, and the striping down of the disease was restricted in most cases to long, narrow areas, with healthy green tissue to either side. In case of the hypodermic injections, however, a width of three to eight or more vascular bundles was involved, i. e., as much breadth of tissue as appeared water-soaked after the injection, but not much more. Even when a great quantity of germs was injected (0.5 cc. or more of a fluid culture) the disease did not appear immediately, develop rapidly, or cause widespread infection of the bulbs.

To show how closely my results tally with those obtained by Dr. Wakker,[1] I will here set down the course of the disease in each of the eight plants first inoculated.

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[1] See Contributions à la pathologie végétale, I, La maladie du jaune, ou maladie nouvelle des jacinthes, causée par le *Bacterium Hyacinthi*. *Archives néerlandaises des sci. ex. et naturelles*, Tome XXIII, pp. 18–20.

Table showing result of inoculations with a pure culture of Pseudomonas hyacinthi (Wakker).

No. of plant.	Date of inoculation, place, manner, and immediate result.	Result of subsequent examinations.					
	Feb. 16, 1897.	Mar. 1.	Mar. 8.	Mar. 13.	Mar. 22.	June 10.	June 23.
I.	First leaf, upper part. Hypodermic. The injection produced a suffused place 1.5 mm. wide and 5 cm. long.	A yellow streak with shrinkage of tissue—confined to the suffused area. Rest of leaf very green and healthy, except 3 small yellow spots where I made ineffectual attempts to insert the needle.	Cut away leaf about 3 inches above bulb and photographed.	No symptoms on base of leaf.	Same as on 13th.	No symptoms. Leaves of plant now dried out except bases, which are yellow-green.	Leaves dried out. Bulb dry and sound-looking. Cut open; sound internally, except one inner scale. One bundle of this scale is bright yellow and is disorganized, being full of a yellow bacterial slime. The disease extends in this one bundle the whole length of scale and spreads out in the plateau, penetrating nearly to base of bulb and covering therein nearly 1 sq. cm. of tissue. Other bundles not examined microscopically.
	Second leaf, upper part. 20 to 30 ordinary needle pricks over less than 1 sq. cm.	Pricked area pale yellow green.	No record......	Tissue dead and thin. The veins are brownish and the parenchyma between is pale yellow or white diaphanous. Disease begins to stripe down a few cm. in one place.	Pricked part dead and yellow-brown. A narrow interrupted stripe extends down 3 cm. and appears again as a very small spot 1.5 cm. lower.		
	Third leaf, upper part. Like No. 2, i. e., 20 to 30 delicate needle pricks.	Pricked area pale yellow-green.	No record......	Decided symptoms over a breadth of 5 mm. and a length of 3 cm., commencing with the pricked area. The punctured part is diaphanous, shriveled, with a feeble browning of the veins.	The terminal 3 cm. of leaf has collapsed and is yellowish-brown. A narrow stripe extends down 2 cm. into the green.		

II.	Leaf, middle part. 20 to 30 needle punctures.	Pricked area pale yellow-green.	No record......	Plain symptoms in the pricked area, with a narrow stripe up and down.	Apex of leaf (4 cm.) brown, shriveled. Diseased stripe rather wide and at least 12 cm. long. All the middle part of the leaf is yellow. Margins green.	Leaves of plant have dried out from age.	Small bulb. Sound externally. Five vascular bundles are bright yellow, gummy, and broken down by bacteria. Four of the yellow bundles are in an outer scale and one is in the next inner scale. The yellow, gummy, bacterial disease extends in these bundles the whole length of the scales and widens out below in the plateau.[1] The rest of the scales are sound.
III.	First leaf, upper part. Hypodermic. The injection produced a water-soaked place 3 to 4 mm. wide and 10 cm. long, i. e., including a breadth of 6 to 7 vascular bundles.	Conspicuous infection. The whole of the suffused area is diseased. The upper part is shriveled, thin, yellowish, and brownish in streaks (in the bundles). The lower part has a water-soaked, pale look, as if it were still suffused from the injection, which is not the case.	No record	A thin, translucent, yellow-brown, dead stripe now extends from the point of inoculation to the apex of the leaf and far down. It is about 5 mm. wide (includes 8 bundles) and 13 cm. long. The stripe is bordered by a narrow band of pale yellow tissue, beyond which, to either side and below, the leaf is a healthy green. No odor. The browning is in the bundles and the cross veinlets, the dead tissue between being pale white or a transparent yellow.	The striping has not reached the base of the leaf. Much as on the 13th, but dried out more. Leaf still green to either side. Side movement of the organism very slow.	Leaves have nearly dried out fom age, except at base.	Bulb dry and sound externally. Leaves have shriveled from age. One vascular bundle in each of 3 scales is bright yellow, broken down, and full of bacterial slime. These bundles are readily traceable into the plateau, where the diseased area widens out. Midway of the diseased scales there are yellow gum pockets surrounding the infected bundles, but the bacteria have not yet escaped into the spaces between the scales.
	Second leaf, upper part. 20 to 30 delicate needle pricks.	Pricked area pale yellow-green.	No record	Marked yellowing of the pricked area, but no death of tissue or diaphanous appearance or down striping of the disease.	Pricked part has dried out and is yellow-brown. No striping down.		

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[1] The solid portion of the bulb, from which roots and scales originate.

Table showing result of inoculations with a pure culture of Pseudomonas hyacinthi (*Wakker*)—Continued.

No. of plant.	Date of inoculation, place, manner, and immediate result.	Result of subsequent examinations.					
	Feb. 16, 1897.	Mar. 1.	Mar. 8.	Mar. 13.	Mar. 22.	June 10.	June 23.
IV.	First leaf, upper part. Hypodermic. The injection produced a water-soaked place .5 to 1 cm. wide and 7 cm. long. In the widest places it covers 12 bundles.	Suffused water-soaked appearance over the whole injected area. By transmitted light this part of the leaf has a pale yellowish green appearance, which is decidedly different from the rest of the leaf.	No record......	The terminal part of the leaf (7 cm.) has dried out. Below this there is a brownish stripe for a distance of 3 to 4 cm. Farther down and on either side of the stripe the leaf is a healthy green.	The narrow brown stripe has extended downward about 4 cm. farther than on the 13th. The leaf is green to either side and below.	Leaves dried out from old age, except bases.	Middle and base of bulb healthy. Two inner scales show traces of the disease in upper part (6 bundles). Barely visible to naked eye.
	Second leaf, upper part. 20 to 30 needle pricks.	Pricked area pale yellow-green.	No record......	Marked yellowing of pricked area, but no shriveling or death of tissues and no down striping.	Pricked area yellow-brown and drying out. Narrow, yellow upward stripe (1 cm.). No down striping.		
V.	First leaf, upper part. Hypodermic. The injection produced a long, narrow, water-soaked place.	Narrow yellow stripe extending 1 cm. below point where the needle entered. No water-soaked places farther down.	No record......	Yellow and water-soaked appearance on leaf for a breadth of 1 to 3 mm. and a length of 13 cm. The stripe now extends from the apex of the leaf halfway to the base of the leaf. In parts of the stripe there is only a very pale browning of the vascular tissue. To either side the leaf is a healthy green.	About as on 13th, but stripe dried out more. It is still narrow; color brown, with a narrow yellow margin.	Leaves dried out nearly to base (from old age).	Bulb dry and sound externally. There are 8 broken-down yellow bundles distributed in 5 different scales. In the middle of the scales two or three of the yellow bundles widen out into gum pockets, but the bacteria have not yet escaped into the spaces between the scales. The disease widens out in the base of the bulb, the plateau being badly diseased.

	Second leaf, upper part. 20 to 30 delicate needle pricks.	Pricked area pale yellow-green.	No record......	Marked yellowing of pricked area, which begins to be transparent. No down striping.	Pricked part is drying out (yellow-brown). Two up stripes; one is narrow and brown, length 1.5 cm.; the other is wide, pale whitish-green, length 5 cm. Also two narrow brownish down stripes 1 and 1.5 cm. long.		
VI.	Leaf, upper part. Hypodermic. Two punctures, resulting in long, narrow, water-soaked places.	A very slight yellowing where the needle entered.	No record......	A pale yellow-green stripe about 2 mm. wide, extending up 1 cm. and down the same distance. No dead tissue.	The leaf now shows a dead stripe some millimeters wide and one decimeter long.	Leaves dried out from old age nearly to base.	One bundle of one outer scale is diseased from top to base, and in the plateau the disease widens out, forming quite a good-sized gummy area. The rest of this scale and all of the others sound to naked eye (not examined microscopically).
VII.	First leaf, upper part. Hypodermic. Result, a long, narrow, water-soaked place.	A long stripe, corresponding to the injected place. Part is yellow and shriveled, while the rest looks much as it did on the day it was inoculated, i. e., water soaked. The stripe is narrow. It extends 2 cm. above the entrance of the needle and 3 to 4 cm. below. The rest of the leaf is turgid and healthy green.	Cut away all but lower 8 cm. of leaf to photograph.	Stub of leaf healthy	No striping down of stub of leaf.	Leaves dead of old age nearly to base.	Only one bundle visibly affected (an inner scale). This is yellow and disorganized above, but the disease can not be detected by the naked eye below the middle of the bulb. Plateau sound.

Table showing result of inoculations with a pure culture of Pseudomonas hyacinthi (Wakker)—Continued.

No. of plant.	Date of inoculation, place, manner, and immediate result.	Result of subsequent examinations.					
	Feb. 16, 1897.	Mar. 1.	Mar. 8.	Mar. 13.	Mar. 22.	June 10.	June 23.
VII.	Second leaf, upper part. Hypodermic. The injection made a long, narrow, suffused streak.	Nothing very definite. Only a narrow yellow stripe 1 cm. long below entrance of needle and a small yellow spot where an abortive attempt was made to enter the needle.	No record	Stripe 1 mm. wide and 2 cm. long. Also, short yellow stripe downward from the abortive puncture.	Stripe at least 16 cm. long. Whole upper part of the leaf flabby and yellow. Leaf green below to either side of the yellow stripe and under it.		
	Third leaf, upper part. 20 to 30 ordinary needle punctures over about 1 sq. cm.	Pricked area pale yellow-green.	No record	Pricked area (12 mm. long and 6 mm. wide) is now dead, shriveled, and transparent—yellow, with pale brown veins. As yet no marked striping down.	Two narrow yellow stripes extending downward 1.5 cm. and 2.5 cm. from pricks. Disease progressing very slowly.		
VIII.	Scape. Hypodermic. Several punctures just under the unexpanded flowers.	Cut away some days since, i. e., as soon as in full bloom. The cut was made 8 or 9 cm. under the points of inoculation and before there were any symptoms.	No record	Plant healthy............	Plant healthy. The leaves of all of these 8 plants have doubled in size since they were inoculated.	Leaves dead nearly to base (from age).	Bulb sound externally. No results were expected, but when cut open 3 of the inner scales showed unmistakable evidence of the yellow disease. On cross section there were 6 small bright yellow spots, corresponding to as many bundles, and from these a gummy bacterial slime exuded. Four of these diseased bundles were in one scale and one in each of two others. The bacteria extended the whole length of the 6 bundles and spread out in the plateau. All the other scales were sound. (*See* Pl. I, fig. 1.)

These bulbs were planted close together in a shallow pot of sandy earth, only the lower half of each bulb being buried. The plants were on a bench in a greenhouse, where there was an abundance of light and air and where they received water from time to time as required. The external symptoms were so slight and progressed so slowly that no record was kept after March 22. The plants were, however, under almost daily observation during April and May. They made a vigorous growth for two months or more after flowering time, but as the warm weather of summer came on the leaves gradually dried out and died from the top down, and, with the exception of the bases, were pretty uniformly shriveled by the middle of June. This shriveling was not, however, the result of the disease. In fact, so little increase of symptoms was observed in April and May that when the bulbs were cut open (June 23) it was with no expectation that any diseased places would be found. That some of the leaf inoculations did dry out and fail after starting was evident, but enough succeeded to place the success of the experiment beyond doubt, one or more bundles in the bulb scales of each one of the 8 different plants being yellow, broken down, and full of bacteria (see Pl. I, fig. 1).

None of these plants became wet-rotten or bad-smelling as a result of the bacterial infection, the symptoms being wholly unlike those obtained by Dr. Heinz with his *Bacillus hyacinthi-septicus*. So far as observed the diseased plants had no odor whatever; certainly no pronounced odor. No mycelium was present in any of these yellow, broken-down bundles, or in any of the bulb scales, and in most cases no micro-organism of any sort was present except the one which had been introduced into the leaves (and scape) in the preceding February; i. e., more than four months before and at a distance of from 15 to 25 cm. from the bulbs. No animal parasites were observed.

Nearly all the scales of these eight bulbs were still entirely sound, but from the condition of the plateau, when the germs had penetrated that far, it was evident that a general infection of the scales and a more or less complete destruction of the bulbs would have been only a matter of time. Even in the attacked scales the greater part of the tissue was still sound.

Previous to making these inoculations I was inclined to attribute the slow progress of the disease in Dr. Wakker's inoculated plants to the low temperature at which his plants were kept, or else to his having used cultures containing very few living germs.[1] Having myself inoculated from a culture in prime condition for experimental purposes (i. e., swarming with motile rods), having in nearly one-half the cases inserted great numbers (that is, thousands) of the germs, having kept the plants at considerably higher temperatures (20° to 30° C.), and yet having obtained the same results as Dr. Wakker, I am forced to the conclusion that the organism is a rather feeble parasite and that the slowness of its progress in the plant is due to natural causes, the discussion of which I will undertake later on.

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[1] From my critical review, published in 1896, it will be seen that even then I was inclined to regard Dr. Wakker's statements respecting pathogenesis as substantially correct, and my subsequent studies have fully confirmed this view.

SERIES 2 (ONION).

On December 13 six shoots of an onion (*Allium cepa*) were inoculated with bright yellow slime from a potato culture (tube 12, December 4), by means of numerous needle punctures.

Result: The plant developed no leaf symptoms, and when the four bulbs (all from one root) were dug and examined in June, 1898, there was no trace of yellow bundles or other indication of disease.

NATURAL INFECTION OF A DAUGHTER BULB.

In April, 1897, a diseased bulb was potted and placed in the hothouse. This was the last remaining bulb of those received from Holland the preceding fall, the rest having been cut for study or having fallen to pieces in the dry air of the laboratory, to which they had been exposed for six months. The planted bulb did not sprout for a long time, but finally developed some feeble leaves. No particular attention was given to it during the summer and fall, but in midwinter I noticed that the leaves were dying at the top and were crooked—i. e., came up exclusively from one side of the bulb and curved over toward the other side. In February, 1898, the plant was knocked out of the pot and examined. The bulb which I had planted was completely decayed. All of the leaves were from a small daughter bulb, which was not present, or at least not visible, when the mother bulb was planted. This bulb was one-sided, had only a few leaves, and these were dying at the top. There was no wet rot of the leaves or bulb and externally the bulb was sound. On cutting it open more than forty vascular bundles in the otherwise sound white scales were found to be bright yellow, and a careful microscopic examination showed them to be full of the hyacinth germ. These yellow bundles were in eight different scales. That the daughter bulb had contracted the disease from the mother bulb which I planted was evident (1) from the fact that there was no other visible source of infection—i. e., this bulb was planted in good soil, in which hyacinths had never grown and was the only hyacinth in the greenhouse; (2) from the fact that the plateau was the most badly affected part of the bulb; and (3) from the fact that the scales seemed to have been infected from below up, the yellow slime in more than two-thirds of the affected bundles being visible to the naked eye only in the lower half of the scales, whereas in bulbs which became diseased as the result of my leaf infections the upper half of the scales (so far as examined) was always the first to show the symptoms. Probably the leaves curved toward the decayed mother bulb from which the infection was received, as in case of one described by Dr. Wakker, but this I neglected to determine.

INOCULATIONS OF 1898.

The following year these experiments were repeated. All the plants were in the same greenhouse. The night temperature of the house for a month or two, during which symptoms were slowly extending in the hyacinth leaves, was 10° to 18° C.; the day temperature was 21° to 31° C. Subsequently, during May and June, the temperature fluctuated more, and some of the time it was considerably higher, especially in the daytime—that is, 10° to 20° C. by night and 30° to 46° C. by day. On quite a good many days during this period the air temperature for some hours ranged from 35° to 40° C.—i. e., too high for the growth of this organism, as shown by maximum temperature experiments, and probably high enough to have been of material aid to the plant in resisting the attack of the parasite.

SERIES 3 (HYACINTHS).

The third series of inoculations was made January 29. Seven well-grown hyacinth plants, not yet in bloom, were selected for this purpose, and eight uninoculated plants were held for comparison. All were inoculated from an alkaline beef-broth culture (No. 4, January 25), using a hypodermic syringe. Two were inoculated by means of numerous punctures into the short, unexpanded inflorescence. The other five plants were inoculated in the apical part of the leaves. The leaves at this time were about one-half grown (10 cm. long), and three on each plant were inoculated. Including what was wasted, about seven to eight cc. of the cloudy fluid was used on the seven plants. These plants were single-flowered and of three sorts—flowers large white with a tinge of blue, flowers large creamy white, and flowers large pink with a deeper stripe down the center of the petals; names unknown. The germs used for this series of inoculations and all of the following were descendants of those isolated in June, 1897, from the yellow, broken-down bundles in the bulbs of the plants inoculated February 16 (see first series).

Result.—Four of the check plants were destroyed by a rapid soft white rot. The other four were sound and free from all trace of the yellow disease when examined June 18.

All of the seven inoculated plants showed distinct above-ground symptoms, the progress of which was slow. Three of these were attacked in the spring by a rapid soft white rot. In one of these, which was dug early, the unsoftened part of the bulb showed two yellow bundles. The other four—i. e., those not attacked by the soft rot, were dug June 18. The bulbs were sound externally. Two were sound internally, so far as could be determined by the unaided eye—i. e., the disease seemed to have died out in the parts above ground. In the other two there were distinct symptoms in the bulb. In one bulb several scales had yellow bundles, and the plateau was also diseased in the upper part; in the other bulb the disease was restricted to two bundles in the upper part of one scale.

SERIES 4 (ONIONS).

Three onion plants (*Allium cepa*) were selected for this series, which was begun January 29, using the same culture medium and method of inoculation as in series 3. Each plant was copiously inoculated in the apex, middle, and extreme base of several leaves.

Result.—The young and tender leaves were killed outright within a few days of the inoculation, with no distinct symptoms of parasitism. The older leaves developed no symptoms whatever, or only such as were due to the slow growth of the parasite in the immediate vicinity of the point of inoculation—i. e., the symptoms were entirely unlike those obtained by Heinz with his *Bacillus hyacinthi-septicus.* In case of half a dozen or more leaves the germ was able to hold its own in the inoculated tissues and finally to make a bright yellow growth in the parenchyma in the vicinity of the punctures. It never extended very far, however, and did not kill the parts in which it grew—at least not until after many weeks. Pl. I, fig. 2, shows the appearance of an onion leaf in which the germ has made a slow growth.

On June 22 these plants were knocked out of the pots and their bulbs examined. Each plant had a good top at this time and was in fruit. One had seven bulbs from a common root, another four, and the third three. All of these bulbs were sound. None showed any trace of yellow bundles.

SERIES 5 (HYACINTHS).

The fifth series of inoculations was made February 7, in the same way as the two preceding. These plants were inoculated from an alkaline beef broth culture (No. 1, Jan. 29), about 0.5 to 0.7 cc. of cloudy broth being used on each plant. Nine vigorous plants in full bloom were selected for this experiment, the variety being a single-flowered, pale-blue sort known as Czar Peter. Two were inoculated in the scape just under the inflorescence (0.3 cc. each, several punctures) and the remainder were inoculated in the apical portion of the leaves, three to seven leaves on each plant being selected for this purpose (generally three leaves).. Twenty-three plants of the same variety and growing in the same box were held as checks.

Result.—Ten of the check plants were attacked by a soft white rot between February 7 and June 14. The bulbs of three of these were only softened a little in places when dug out and these bulbs showed no trace of yellow bundles. The other seven were destroyed by the rot. None of the remaining thirteen check plants contracted any disease and their bulbs were sound and entirely free from yellow bundles when cut open and examined on June 14.

Two of the inoculated plants were also attacked by the same rapid soft-rot.[1] The bulb of one, which was left undisturbed, finally decayed completely; that of the other was pried out February 14 to prevent the spread of the disease. At this time there were no foliar symptoms due to the inoculations. The soft-rot had just begun. It started at the base of two leaves in wounds accidentally made by the knife of the gardener in cutting away the scape.

Distinct symptoms of the yellow disease appeared on the above-ground parts of each of the eight other inoculated plants, and progressed slowly in the usual way. The notes upon plant No. 25, given below, will answer for all. On June 14 the bulbs of these plants were examined. One was free from bacterial infection so far as could be determined by careful cutting and microscopic examination. One was rotted and gone, as already noted. The six other bulbs were sound externally but, within, each one showed distinct symptoms of Dr. Wakker's disease—i. e., there were few to many yellow bundles full of bacteria in otherwise sound scales. In most cases the plateau was also involved. Generally the yellow disease was closely restricted to individual bundles, the parenchyma between them being sound. In several cases, however, small bacterial pockets had formed in the parenchyma around a bundle; in one case all of the parenchyma between two neighboring bundles was yellow; rarely, some of the smaller anastomosing veins would be yellowed nearly to a neighboring bundle. All of these features are shown in fig. 1 (from plant No. 20). In no case was there observed any rupture of the epidermis of the affected scales or flow of the yellow slime between the scales, the bulbs being examined too early for this stage of the disease.

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[1] This parasite, which is a rapid-growing, bad-smelling, actively-motile white germ, probably identical with *Bacillus hyacinthi-septicus* Heinz, came from the hot house where the bulbs were forced. The box originally contained 35 bulbs, the rotten, sour-smelling remains of 3 being discovered and pried out after its purchase. All the plants inoculated in 1898 came from this same forcing house and nearly every pot or box developed some cases of this disease. Otherwise the plants were very satisfactory.

This organism was not studied critically, for lack of time, but some notes were made. The bacterial slime and accompanying tissues of the host plant taken from the upper inner part of a diseased scape (the advancing margin of the decay) were examined microscopically. There was no mycelium or insect injury, and the innumerable bacilli were apparently all one thing. The rods were 2 to 5 μ long, and rather less than 1 μ broad, with rounded ends. They were single or in pairs. Very few were in motion at first (the slime was diluted with a drop of distilled water), but within a few minutes many became actively motile. This motion consisted mostly of rapid movements straight ahead, and often straight back in the same track, for a distance many times the length of the rod. Tumbling and sinuous movements, however, were also observed. Toward the close of the first hour at least one-fourth of the rods were in motion. In form, the motile ones were exactly like the others. While watching, I frequently saw stationary rods become motile and dart away. These rods stained readily in basic fuchsin water and in gentian violet water. This slime from the host plant gave a faint bad smell and was slightly sticky, stringing up 1 centimeter. Cultures made directly into tubes of potato from the same part of this scape, after cutting it open with a burning hot knife, yielded a rather slow-growing, not very copious, wet-looking, smooth, white slime, which was strongly alkaline and somewhat sticky, stringing up 1 to 3 centimeters when touched with the loop. The four potato cultures were alike at first and three continued to be homogeneous, while a pink organism appeared in the fourth tube at the end of the second day. A few gas bubbles also appeared in each of the tubes.

This particular hyacinth plant was a robust Czar Peter in full bloom, with a long stocky scape. The rapidity of the rot may be judged from the fact that when the disease was first discovered it involved only one flower. In forty-eight hours the scape was soft-rotten (and lopped over) from the point of infection nearly to the bulb (10 or 15 centimeters) and also 3 to 5 centimeters above the point of entrance—i. e., to within a few centimeters of the top of the inflorescence. It was a soft wet rot, involving all of the tissues in a general collapse of slime which was strongly alkaline. Another fact worthy of note is that this organism is quite tolerant of acids.

That we have here a genuine bacterial disease of the hyacinth, worthy of careful study, admits of no doubt whatever.

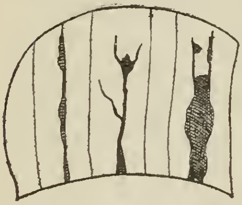

Fig. 1.—Diseased scale of hyacinth.

Notes on No. 25.—Inoculated February 7 in the apical part of 5 leaves by means of a hypodermic syringe, suffused stripes resulting in each case.

February 14. Leaves 12.5 centimeters long. No symptoms. The suffused stripes due to the injection soon disappeared. The absence of symptoms is surprising, considering the quantity of germs inserted and the time that has elapsed (seven days).

March 1. Each of the five leaves now shows a yellow stripe down its center. The breadth of these stripes is 3 to 6 mm. A few of them extend from near the tip of the leaf almost to its base. Below the shorter stripes is a line of narrow, interrupted, water-soaked spots. To either side of the stripes the leaves are green and normal in appearance. On one leaf only has any tissue shriveled, and that to but a small extent. The parenchymatic tissue in the stripes has become translucent, while the parallel main bundles begin to be feebly browned. The greater part of each leaf is still healthy, the symptoms being confined to the vicinity of the injected parts. The symptoms a week ago (fourteenth day) were very slight.

April 30. A marked increase of symptoms. The stripes now extend one-half way down, two-thirds down, and entirely down to the base of the leaf. The parts which were striped on March 1 are now dead and diaphanous or brownish. The deepest brown is in the larger vascular bundles, and is feeble in comparison with the brown veining of the cabbage produced by *Ps. campestris*. At the base of these dead stripes the disease continues in the form of water-soaked stripes, which are more or less interrupted, i. e., the surface symptoms disappear and reappear a few millimeters lower. To either side of the dead, brown stripes there is a narrow yellow line beyond which the tissue is green and normal in appearance. Two of the leaves have collapsed and dried out at the tip (1 cm. and 6 cm.). The slow sidewise movement of the disease is very marked, and becomes astonishing when we consider the enormous number of germs originally inserted into these leaves. On one of these leaves there is an interrupted, water-soaked stripe in the narrow yellow border, indicating a recent slight sidewise movement of the parasites. All of these symptoms are shown in fig. 2.

June 14. The leaves are dead; the bulb is sound externally. On sectioning, the interior of the plateau was found to be diseased and there were also twenty-two yellow bundles distributed through eleven different scales. These yellow bundles were partially broken down and full of bacterial slime. That many of them were tertiary infections (from the inoculated leaves by way of the plateau) was very plain, since the yellow slime, while always distinct in the basal part of the bundle next to the plateau, frequently became less abundant or disappeared altogether in the middle or upper part of the scale. The greater part of the bulb was still sound. No mycelium was present and there were no injuries from animals.

SERIES 6 (ROMAN HYACINTHS).

Four Roman hyacinths (*Hyacinthus albulus*) were selected for this series, which was started February 7. The plants were fine specimens, in full bloom. Two of them were inoculated in the middle part of the scapes (three scapes on each plant) and the other two in the apical part of the leaves (four leaves on one plant and seven leaves on the other). The infectious material, alkaline beef-broth cultures (Nos. 1 and 4, January 29), was put in by means of a hypodermic syringe. Two plants in the same pot were held as checks.

Result.—The six inoculated scapes gradually shriveled, but with no symptoms clearly attributable to the action of the germs. Each of the eleven inoculated leaves slowly developed narrow stripes corresponding to the parts of the leaf suffused at the time of the inoculation. These stripes did not appear until after the seventh day. There was very little sidewise extension, and the downward movement was very slow. At first the stripes presented a water-soaked appearance. Later they were pale yellow, with brownish veins, and when dead and dry they were yellow-brown. No such stripes appeared on any of the uninoculated leaves, of which there were many. On June 15, when all the leaves were dead and gone, the bulbs were removed from the pot and examined. Each had formed several to many daughter bulbs, but neither in these nor in the mother bulbs was there any trace of the yellow disease. All were sound so far as could be determined by the unaided eye.

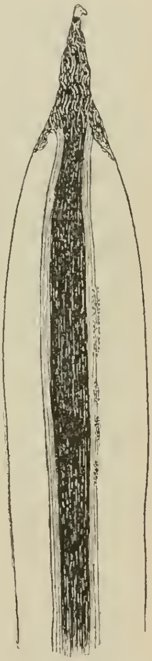

FIG. 2.—Inoculated leaf of hyacinth No. 25.

SERIES 7 (HYACINTHS).

The seventh series of inoculations was made February 7, in the same manner as the preceding. For this experiment I selected sixteen vigorous plants of the single, white-flowered Baron van Tuyll, holding fifteen plants of the same variety growing in the same box as checks. The plants were in full bloom. Each bore five to seven good leaves, three of which were inoculated in the apical part. Each plant received from 0.5 to 0.7 cc. of the cloudy alkaline beef broth culture (No. 4, Jan. 29).

Result.—Thirty-five bulbs had been planted in this box. Two were rotted and gone when it was purchased, and a rapid soft rot developed on the scapes of two others a few days later, so that only thirty-one healthy plants remained at the date of inoculation. The fifteen plants held as checks never developed any leaf symptoms comparable to those on the inoculated plants, and fourteen of the bulbs were entirely sound when dug and examined June 16. The other bulb was free from the yellow disease, but was just beginning to succumb to the soft white rot (the extreme top of the bulb).

Every one of the forty-eight inoculated leaves (sixteen plants) developed symptoms of the yellow disease. These symptoms appeared for the most part only after fifteen to thirty days, and the progress of the disease was very slow, although distinctly visible for a month or two, i. e., until the hot weather set in, when the disease seemed to die out in many leaves. On June 16, when the bulbs were dug and examined, two were soft-rotted, with the exception of a few outer scales, which showed no trace of the yellow disease. The other fourteen plants had better-preserved leaves than the corresponding plants of Czar Peter (series 5). Ten of the bulbs were entirely free from symptoms of the yellow disease and perfectly sound so far as could be determined by the unaided eye. The other four were attacked by the yellow disease, but not extensively, and for the most part only in those scales which bore the inoculated leaves. All of this variety took the disease less rapidly than the Czar Peter. The plants were very carefully examined from time to time and notes made on the condition of each one, the two sets of notes which follow being fairly illustrative of the whole lot.

Notes on plant No. 40.—Inoculated February 7 in three leaves.

February 14. No symptoms. The plant has five leaves which are now 17.5 cm. high.

March 1. One leaf only shows any decided symptoms. These consist of a stripe in the upper central part of the leaf (the inoculated part) which is yellow in the wider upper part of the stripe and water-soaked in the lower 3 to 4 cm. The length of the stripe is 9 cm., the breadth is 2 to 3 mm. in the upper part and only 1 mm. in the lower, water-soaked part. Symptoms in the other inoculated leaves are restricted to the vicinity of the needle puncture, and consist of a slight water-soaked appearance in the form of narrow, short, interrupted lines. All of this white variety have taken the disease less rapidly than the Czar Peter.

March 30. There has been a distinct progress of symptoms. On the first leaf there is a stripe of yellow-brown, dry tissue 3 mm. wide and 7 cm. long. On the second leaf there is a stripe 3 to 5 mm. wide and 3 cm. long, which is yellow with a dry, brown center. On the third leaf the stripe is 5 mm. wide and 3 cm. long. Most of this is simply yellow, but the central part is dry and brownish. Below the well-defined stripe are narrow, short, interrupted water-soaked lines on a green background. These water-soaked lines are separated from the older yellow-brown stripe by 3 to 4 cm. of healthy-looking tissue. This appearance must be due to germs which have broken out of the bundles and grown or diffused into the parenchyma in these particular places, or which have so destroyed the tissues as to allow the juices to flow out. The greater part of the three inoculated leaves and all of the other two leaves are sound. The leaves are now about 30 cm. long. The opinion of March 1 as to the greater resistance of this variety is certainly confirmed by the observations made to-day.

June 16. The basal 15 cm. of two of the inoculated leaves is sound. On the third it is sound, except for a narrow, interrupted, water-soaked stripe which extends to within 5 cm. of the bulb. The bulb was carefully sectioned at various levels from the base of the plateau to the top of the scales, but there was no trace of yellow bundles or any other symptom of disease. Of course it does not follow that some of these bacteria had not gained entrance to the underground parts or that six months later this bulb would not have been diseased. Indeed, I believe it would have been.

Notes on plant No. 46.—Inoculated in apical part of three leaves on Februry 7.

February 14. No symptoms. The plant has five leaves, 17.5 cm. long.

March 1. Long, narrow, water-soaked lines have appeared in the injected part of two of the inoculated leaves. As yet there is no yellowing. The third leaf shows no symptoms.

March 30. There are now distinct symptoms on each of the inoculated leaves. The stripe on the first leaf is 5 cm. long and 3 to 5 mm. wide. It is brown in the upper (widest) part, where the bulk of the injected fluid must have lodged. The tip of the second leaf is dry and brown (3 cm.), and in the middle of the leaf from this point down for a distance of 8 cm. the symptoms continue in the form of narrow, interrupted, water-soaked stripes. On the third leaf the yellow stripe is 5 mm. broad in its upper part and 1 to 2 mm. wide in the middle and lower part. Farther down the stripe is composed of narrow, interrupted, water-soaked lines on a green background. No part of the stripe is brown. The rest of the plant is normal. Here, as in No. 45, the symptoms on one leaf did not develop until after twenty-one days, and from the present appearance probably not until more than thirty days had passed. This is very remarkable, considering the number of germs used, and can be explained only on the supposition that most of them have been destroyed in the plant or, if not killed outright, have been able to overcome retarding influences only very slowly.

June 16. The basal 5 to 15 cm. of each leaf is sound externally; the rest is dead and dry. The bulb is sound externally. On cutting open, one scale only was visibly affected. This scale bore one of the inoculated leaves, and the visible symptoms were restricted to the upper third of the bulb and to one bundle. The plateau and all the other scales were free from symptoms, but probably a careful microscopic examination would have shown the beginning of disease in other bundles of this scale.

SERIES 8 (HYACINTHS).

The eighth series of inoculations was made February 11 in the same manner as the preceding. For this experiment two plants of the variety known as Gertrude were selected, and two plants of the same variety, in the same pot, were held for comparison. This variety is a deep-rose, single-flowered, vigorous-growing sort. The plants were in full bloom. One had eight leaves, the other nine. Three leaves on each plant were inoculated near the apex from the well-clouded beef-broth culture (No. 6, Feb. 5), 0.3 cc. being injected into each leaf. These leaves were 10 to 12 cm. long. The needle was inserted about 2.5 cm. from the apex of the leaf, and the narrow, suffused (water-soaked) stripe which appeared immediately after the injection of the fluid often extended nearly to the base of the leaf.

Result.—Characteristic symptoms of the yellow disease were visible on each of the inoculated leaves as early as February 14. At first the disease progressed much more rapidly than on any other variety. Later its spread was slow. The stripes in the inoculated leaves extended downward slowly until the end of March, at which date there were symptoms on no other leaves. About this time both of the inoculated plants and the two check plants were attacked and destroyed by the rapid soft white rot.

SERIES 9 (HYACINTHS).

The ninth series of inoculations was made February 11 from the same culture and in the same manner as the preceding, i. e., 0.3 cc. of the cloudy fluid was injected into each leaf. For this experiment I selected two healthy plants of a single-flowered, pale-rose variety known as Gigantea. Six plants of the same variety in the same pot were held as checks. The plants were in full bloom. Each plant had four to five leaves, three on each plant being inoculated near the apex. At this time the leaves were 9 to 11 cm. long.

Result.—On February 17 there were no symptoms on either plant. By March 1 there were pronounced stripes on four of the six inoculated leaves. The other two leaves (seventeenth day) showed no symptoms. These stripes were 2 to 3 mm. wide and 6 cm. long, extending down the middle of the leaf. The older portions of these stripes were dull yellow and semi-transparent, with pale brown bundles. Above and below this portion the striping continued in the form of water-soaked spots. To either side of these narrow stripes the leaf was healthy. The appearance of one inoculated leaf from each plant (March 5) is shown in Pl. I, figs. 3 and 4. Later, both of these plants were spoiled by the rapid soft white rot. None of the uninoculated leaves ever showed any symptoms of the yellow disease. Two of the check plants developed the rapid soft rot and were dug out soon after the experiment began. In both the rot began in the blossoms, and in one it was still confined to a single flower and a small portion of the adjacent scape when discovered. The other four check plants were dug and examined June 17. All were soft-rotted at the heart, but in the scales which remained in condition to be examined there were no yellow bundles.

SERIES 10 (HYACINTHS).

The tenth series of inoculations was made February 11 from the same culture as the preceding. For this experiment another pot of Gertrude was selected. The plants were in full bloom and very healthy. Four were inoculated and four others in the same pot were held for comparison. Each of the plants bore eight to ten leaves. Two were inoculated in the apical portion of the leaves (three leaves on each plant) by means of a hypodermic syringe, 0.3 cc. of the culture being put into each leaf. The other two were inoculated in the same way in the scape, just under the truss of flowers, several punctures being made. One of these scapes received 0.3 cc. of the cloudy broth and the other 0.6 cc.

Result.—Four of the six inoculated leaves showed distinct symptoms on February 14. No stripes were visible on the other two until after February 17, and they were slight on March 1, consisting merely of some narrow, parallel, water-soaked lines. On March 1 two of the other four leaves were shriveled to the base, and a third was shriveled halfway down and showed water-soaked places farther down. Neither of the plants inoculated in the scape showed any symptoms until after February 17, all of the flowers wilting normally. On March 1 the scape which received 0.3 cc. showed one very narrow, short, water-soaked stripe in the upper part under the shriveled flowers, and at the end of this month some of the leaves began to be yellowish-green between the vascular bundles as if disturbed in their nutrition. The scape which received 0.6 cc. showed on March 1 two or three narrow, short, water-soaked lines below the shriveled flowers. At the end of this month the scape was wholly shriveled and the leaves dead at the top (upper 3 to 6 cm.). On June 17, when the bulbs were dug for examination, all were spoiled by the soft rot.

The leaves of the check plants never developed any symptoms of the yellow disease. On June 17, when the bulbs were dug for examination, all of them were soft-rotted at the heart, but none of them showed any trace of yellow bundles.

SERIES 11 (CABBAGE).

The eleventh series of inoculations was made on young cabbage plants in active growth. They were inoculated February 11 from the same culture as the preceding (No. 6, February 5). On each of two plants the germs were forced into several parts of two leaf blades by means of the syringe, and on each of the same leaf blades numerous delicate punctures were made with the tip of the needle and the fluid bearing the germs was carefully rubbed in and not allowed to dry immediately. To prevent any injurious action of sunshine or of dry air large drops of the culture were finally put on the punctured parts and sheltered from the direct action of the light and of air currents until nightfall. The germ-laden fluid was forced into 2 petioles of a third plant, so that they showed long, suffused streaks, while here and there the fluid oozed through the epidermis in many very tiny drops. The blade of a third leaf on this plant was punctured, inoculated, rubbed, covered with fluid, and sheltered as described above.

Result.—After some days the two injected petioles split open, but no other symptoms appeared, not even in the immediate vicinity of the injected and punctured parts. The plants were under observation nearly four months, and differed in no respect from the check plants. Inoculations of such plants with *Pseudomonas campestris* led to very different results, as I have shown elsewhere.[1]

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[1] See Centralb. f. Bakt., 2. Abt. Bd. III, July, 1897, p. 284.

SERIES 12 (AMARYLLIS).

The twelfth series of inoculations was made February 11 on the well-grown leaves of *Amaryllis atamasco*. Two plants, not yet showing any flower shoots, were selected for this purpose, and three healthy plants in the same pot were held as checks. Two leaves were selected on each plant and 0.3 cc. of the cloudy broth culture (No. 6, February 3) was injected into each of two places on each leaf.

Result.—The symptoms developed very slowly as feeble yellowish stripes, confined to the parts originally suffused. Subsequently in the striped part the plants produced, as is their wont when injured, a red pigment. On March 1 this red pigmentation was quite distinct in each one of the eight inoculations. On March 31 it was more pronounced, occurring mostly in the form of interrupted red streaks on a green background. These were visible on one leaf for 10 cm. below one of the needle punctures and for 12 cm. above the other. On another leaf the red dots and stripes extended 7 cm. above a needle puncture and 9 cm. below it. At this point there were more than 100 red dots, each less than 0.5 mm. in diameter. These red spots were in parallel rows over vascular bundles and not in the parenchyma between the bundles. In the widest part of the stripe four vascular bundles had these red spots over them. In the oldest and worst stained part of the stripe (near the puncture) the red stain also involved the parenchyma between the bundles. After this date the disease made only very slow progress. On June 18 the bulbs were knocked out of the pot, sectioned at many levels, and carefully examined. All were entirely free from any trace of yellow bundles and perfectly sound.

SERIES 13 (HYACINTHS).

The thirteenth series of inoculations was undertaken February 12, 3 p. m., to determine whether infections might not be secured through the blossoms. For this purpose I selected four single, blue-flowered, healthy plants of Baron van Tuyll, four plants of the same variety and in the same pot being held as checks. All were in full bloom. Six flowers on each of the four plants were inoculated by putting a big drop of cloudy beef broth (No. 11, February 3) gently into the throat of each one without in any way touching the flower with the needle of the hypodermic syringe. The pot and the earth on which it stood were heavily watered and then covered with a large bell jar. This jar was removed February 14, at noon, when the drops had disappeared. Bees had access to the hothouse and visited these plants freely all day, but for the most part they carefully avoided the inoculated flowers. In one instance, however, I saw a bee enter an inoculated flower. Frequently they passed in front of such flowers and occasionally prepared to enter and then suddenly withdrew and selected uninoculated flowers.

The throat of the contracted perianth did not wet readily, and so much uncertainty was felt as to likelihood of the infection reaching the nectaries that this series and the following one were repeated. (See series 16 and 17.)

Result.—These plants were examined March 2 and again March 31. On three of them there were no symptoms whatever and on the fourth there were symptoms of ill health, but none clearly attributable to the inoculation. (The bulb of this plant subsequently rotted.) On June 18, when these plants were next examined, one of the bulbs had soft-rotted, two appeared to be sound, and the fourth was affected with the yellow disease. My notes on this particular plant are as follows·

Notes on plant No. 63.—February 12, 3 p. m. Inoculated six flowers.

February 14, noon. Bell jar removed. The fluid has disappeared from the flowers.

February 17. The flowers are still in good condition. The inoculated ones have not shriveled or fallen off.

March 2. The scape is green and healthy to its tip. There is no evidence of any infection. The flowers have shriveled, but it is a normal withering. The leaves are sound. They are 30 cm. long and the scape is somewhat taller.

March 31. The leaves are healthy and there is no sign of yellowing, shriveling, or down-striping in the scape, which is still green and perfect to its summit.

June 18. Leaves dead, bulb sound externally except for a slight dry rot in the extreme outer part of the plateau, which entirely disappears 1 mm. in. Considerably farther up, the bulb shows distinct symptoms of the yellow disease, which increase in the plateau from below upward. In the upper part of the plateau there are quite a number of yellow bundles and several small cavities full of yellow bacteria. Near the plateau twenty-three vascular bundles in eleven scales are yellowed and more or less broken down by the bacterial slime. Farther up (near the top of the bulb) only sixteen bundles are visibly affected. These are close together on one side of the bulb in eight scales (Plate I, fig. 5). One scale of this bulb was photographed by itself and is shown in Plate I, fig. 6.

None of the check plants showed any symptoms of this disease. On June 18 three of them were entirely sound, while the fourth was partly destroyed by the soft white rot.

Infection of a daughter bulb.—On the flat side of the bulb (shown in Plate I, fig. 5), and still attached to it by a common plateau, was a good-sized daughter bulb. This was also diseased, but only where it joined the mother bulb. In the base of its plateau there were 20 vascular bundles full of the yellow slime, but the upper part of the plateau showed no symptoms and all of its scales were sound.

SERIES 14 (HYACINTHS).

This series was begun February 12 and was in all respects a duplicate of the thirteenth, except that a large single, white-flowered variety, known as Mont Blanc, was used. Six flowers on each of five plants were inoculated and three plants in the same pot were held for comparison.

Result.—Up to March 31, at which date the observations ceased, there were no symptoms on any of these plants which could be definitely ascribed to the inoculations. On June 17, when the bulbs were dug and examined, the three check plants were entirely sound. Three of the inoculated plants were also sound, or at least appeared so to the unaided eye. The bulbs of the two other plants were sound externally, but on sectioning them they showed unmistakable symptoms of the yellow disease. One was slightly affected in two scales. The other was more seriously diseased, as will be seen from the following account of it:

Notes on plant No. 67.—February 12, 3 p. m. Inoculated six flowers.

February 14, noon. Removed the bell jar. The heart of the inoculated flowers is still moist.

February 17. The flowers begin to shrivel. The inoculated ones are holding up best.

March 2. The flowers have withered. The scape is large and 30 cm. long. Its upper 2 cm. is yellow and shriveling, but there are no symptoms attributable to the inoculations. The rest of the scape is green and turgid. The leaves are 20 cm. long and are healthy.

March 31. The scape has dry-shriveled and all of the leaves are drying out at the tip (3 to 10 cm.). The plant looks bad, but there are no stripes on the leaves, not even at their extreme base.

June 17. Leaves dead, bulb sound externally. On cutting, twenty-two yellow bundles were found in the upper part of the white and otherwise sound plateau. The infected bundles were all on one side of the bulb and were beautifully distinct, as in case of No. 63. In the upper part of the bulb eleven bundles in four scales were visibly affected, the yellow slime oozing from the cut surface. Lower down (near the plateau) a larger number of bundles were yellow, and one other scale was involved (one bundle, in which the yellow disappeared about halfway up). The extreme base of the plateau was sound, and, as in No. 63, the progress of the infection was clearly from the scape to the vessels of the plateau and from the latter to the scales. There was no soft white rot.

SERIES 15 (ONIONS).

The fifteenth series of inoculations was made February 12 on *Allium cepa.* Four well-grown onion plants not yet in bloom were selected for this purpose. The inoculations were by means of a hypodermic syringe, using the well-clouded beef broth in tube No. 11 (February 4). About 2 cc. was injected into one plant, numerous punctures being made into old and young leaves. Three leaves were selected on each of two other plants and 0.3 cc. was injected into the base of each one. The fourth plant was inoculated in the same way, 0.3 cc. being injected into the base of each of four leaves.

Result.—On March 2 the inoculated leaves, in whole or in part, were shriveled and white. On March 31 there were no additional symptoms. On June 18, when the bulbs were dug and sectioned, all were free from yellow bundles and entirely sound.

SERIES 16 (HYACINTHS).

The sixteenth series of inoculations was made February 16, at 11 a. m. Two single, white-flowered hyacinths, of the variety known as Baron van Tuyll, were selected for this purpose and two plants of the same variety, and in the same pot, were held for comparison. The interior of eight to ten flowers on each plant was thoroughly infected by forcibly spurting 0.2 cc. of cloudy alkaline beef broth (from tube 3, February 10) into the throat of the perianth. Great care was taken not to spill any of the culture on the leaves or to wound the flowers with the tip of the needle. The pot was wet down thoroughly, covered with a bell jar, and shaded from the light. After twenty-three hours the bell jar was removed, the interior of the injected flowers being still moist. The plants were in full bloom and very thrifty.

Result.—On March 2 one scape showed a trace of water-soak in the part occupied by the flowers, but there were no additional subsequent symptoms. On June 21 these bulbs were dug and examined. Neither one showed any trace of the yellow disease. The two check bulbs were also sound. This variety took the disease slowly in series 7.

SERIES 17 (HYACINTHS).

This series was in all respects a duplicate of the preceding, except that I used two single, blue-flowered specimens of Baron van Tuyll, and inoculated a third plant in the leaves, holding two healthy plants in the same pot as checks. On February 16 eight to ten flowers were inoculated on each plant, each receiving 0.2 cc., which was spurted into the depths of the perianth, where it remained in foam. Of the plant inoculated through the leaves, one leaf received 0.4 cc. and the other two leaves 0.2 cc. each. Each foliar inoculation was made well toward the apex of the leaf.

Result.—On March 2 one of the two plants inoculated in the flowers showed distinct symptoms in the scape. These consisted of a water-soaked stripe beginning in the middle part of the inflorescence in one of the inoculated flowers. The stripe extended downward about 5 cm. and involved about one-third of the circumference of the scape. In the upper part of it the vascular bundles were feebly browned (Pl. I, fig. 7). The disease moved downward rapidly in the scape, and on March 31 the soft white rot having set in, the bulb was dug and examined. There was some yellow slime in the plateau, and one bundle of one scale was visibly invaded by the yellow microorganism. The part of the bulb recently invaded by the soft white rot was the upper central part, i. e., that previously injured by the growth of the inoculated organism. Up to March 31 the other plant developed no symptoms on the scape or leaves, but the bulb was wholly decayed when dug and examined June 17. The cause of this decay was not then determinable.

The plant inoculated through the leaves developed beautifully typical water-soaked stripes down the middle of each leaf. On March 2, two of these stripes were over 10 cm. long. On March 31 the inoculated leaves were shriveled over halfway to the bulb. This plant was not again examined until June 17, when the bulb was wholly decayed and the cause of decay not determinable. The two check plants never developed any above-ground symptoms, and on June 17 the bulbs were entirely sound.

SERIES 18 (PLUNGE EXPERIMENT).

The eighteenth series of inoculations was made March 10, to determine whether infections could be obtained through the stomata. For this purpose I selected six pots of healthy hyacinths of the following varieties: Czar Peter, Gertrude, and Gigantea. All were in full bloom.

The material for infection consisted of 1,000 cc. of distilled water, sterilized in the ordinary way after adding 10 cc. of alkaline beef broth. When sterile, a well-developed beef-broth culture of the hyacinth germ was poured into this flask, the fluid in which was feebly clouded next morning and swarming with motile rods. On plating out, it proved to be a pure culture of the hyacinth germ. Sterile tumblers were filled with this fluid and the apical part of the leaves of selected plants were plunged into it as follows, and left twenty-three hours shaded from the light. On removal, the fluid adhering to the leaves was carefully dried in situ by exposure to the sun before the plants were left, great care being taken not to infect other parts of the same plants or of the checks.

Notes on plant No. 81.—One plant of Czar Peter, six leaves plunged 4 to 7 cm.; three healthy plants in same pot held for comparison.

March 20. No results.

March 30. Plunged part of three leaves is paler green, and one of them has a long, narrow, brown stripe. This is 13 cm. by 1 mm., and begins 0.5 cm. below the tip.

June 21. Leaves dead, bulb sound, at least to unaided vision. All of the check bulbs are free from the yellow disease and all are sound, except the outer part of one plateau, which has soft-rotted.

Notes on plant No. 82.—One plant of Czar Peter, four leaves plunged 4 to 8 cm.; three healthy plants in the same pot held for comparison.

March 30. No visible symptoms.

July 1. Bulb entirely soft-rotted. One of the check plants has also entirely soft-rotted. The other two are sound.

Notes on plant No. 83.—One plant of Gertrude, eight leaves plunged 3 to 6 cm.; seven healthy plants in the same pot held for comparison.

March 30. For the last ten days one leaf has been curved downward in the plunged part, and this part now bears alternating narrow green and yellow stripes, the latter lying in the parenchyma between the bundles. One other leaf shows slight geotropism in the plunged part and slight yellowing in stripes between the bundles. The others are normal.

July 1. The leaves are gone. The bulb has lost its center by soft rot. The scales which remain show no trace of yellow bundles. The checks were also examined. Two bulbs are sound. One is white-rotted and soft on one side, but shows no trace of the yellow germs. The other four are entirely soft-rotted and gone.

Notes on plant No. 84.—One plant of Gertrude, eight leaves plunged 3 to 5 cm.; six healthy plants in the same pot held for comparison.

March 30. No result.

June 21. Bulb rotted and gone. Of the six checks one is entirely sound, three are slightly soft-rotted, but with no trace of yellow bundles, and two have entirely decayed.

Notes on plant No. 85.—One plant of Gigantea, four leaves plunged 3 to 7 cm.: six healthy plants in same pot are held for comparison.

March 30. No symptoms.

July 1. The bulb has rotted, and it is too late to determine the cause. The bulbs of all the check plants have also rotted.

Notes on plant No. 86.—One plant of Gigantea, five leaves plunged 2 to 7 cm.: three healthy plants in the same pot were held for comparison.

March 30. No symptoms.

July 1. Leaves dead. Heart of bulb rotted out. No symptoms of the yellow disease in the scales which remain. The checks are also free from this disease. One of them has soft-rotted. The centers of the other two are also soft from the presence of the white rot.

INOCULATIONS OF 1899.

SERIES 19 (HYACINTHS).

This experiment, begun February 22, was another attempt to infect through the blossoms. From 4 to 10 flowers were inoculated by putting several small drops of the infectious fluid into the heart of the blossoms by means of a sterile hypodermic syringe. For infection, I made use of slime from an actively motile young bright-yellow culture on coconut. This slime was dissolved by shaking in a small quantity of distilled water.

The varieties tested were Regulus,,blue Baron von Tuyll, white Baron von Tuyll, Gertrude, and Gigantea.

The experiment was unfortunately interrupted on June 7, at which time the bulbs of 8 of the inoculated plants were visibly affected by the yellow disease, i. e., about one-third of the whole number. About 40 plants were held as checks, none of which showed any external or internal symptoms of the disease. Regulus was affected to a greater extent than the others, but in all cases the symptoms were slight, and some months more would have been necessary for the bulbs to become seriously diseased.

REMARKS ON PATHOGENESIS.

The inoculation experiments were all made with pure cultures, on sound plants, in a hothouse where hyacinths had never before been grown, and in a country where the disease is not known to occur. Moreover, none of the several hundred check plants contracted the disease. It is therefore reasonably certain that all of the infectious material was derived from my cultures. The pathogenic nature of these cultures is rendered certain (1) because the symptoms always began in that part of the plant which was inoculated and proceeded downward, the bulb being the last part to show the disease; (2) because the organism occurring so abundantly in the yellow bundles of the bulbs was demonstrated by cultures therefrom and by microscopic examinations to be the same as that inserted into the leaves and scapes months earlier; (3) because, after cultivation on artificial media for a year, this organism again produced the disease when inserted into the leaves and floral organs of healthy plants, and, after a lapse of some months, was again demonstrated to be present in enormous numbers in yellow broken-down bundles in the interior of the bulbs.

The time of first appearance of symptoms in the inoculated leaves varied within wide limits, according to the variety tested and the amount of material used, but nearly all the specimens of *Hyacinthus orientalis* which were inoculated showed the disease in three to thirty days in the parts above ground, and 40 of these plants also showed characteristic symptoms in the bulbs at the end of two to five months. In 1898 the conditions toward the end of the experiments were very unfavorable to the progress of the disease, owing to the extreme heat of the summer. In 1899 the experiments were disturbed and broken off too soon.

The results I have obtained indisputably confirm Dr. Wakker's statements respecting the ætiology of this disease. My studies lead me to accept substantially all of his statements regarding the character and succession of symptoms in this disease and the lesions in the host plant due to its progress. They seem to show that some varieties are more susceptible than others, e. g., Czar Peter than white Baron von Tuyll, and Gertrude than Gigantea. They show, as Wakker stated, that daughter bulbs contract the disease from mother bulbs. They do not clearly establish that the germ has any other host plant or that the parasite can enter through the stomata. They show that it is easy to induce the disease by wounds. They also indicate that bulbs may sometimes become diseased as the result of germs lodged in the flowers, and that bees sometimes visit such flowers. The last two facts point to leaf-eating and nectar-sipping insects as probable carriers of this disease. A priori, there is nothing improbable in this view, since two bacterial diseases common in the United States, the cucurbit wilt and the pear blight, are disseminated in this way, the former from germs lodged in the leaf, principally by the bites of leaf-eating beetles, the latter from germs lodged in the nectaries by bees and other insects which visit the flowers for nectar and pollen. It remains, however, for some one in the Netherlands, where the bulbs are grown in quantity, and where the disease is prevalent, to remove this statement from the domain of likelihood to that of actual fact or to show that it has no real foundation.

Wakker believed the disease to be often transmitted by the knife, and there is every reason to think his views well founded. In this case the practical deductions are easily made. Knives used on diseased plants should not be used on healthy plants until they have been thoroughly disinfected. For this purpose it is only necessary to dip them into boiling water for a few minutes.

Possibly healthy fields may become infected from the slime of the canals, into which, I am told, diseased bulbs are commonly thrown and from which the fertile mud is raked out at stated intervals to spread over the land. From the close resemblance of this germ to *Ps. campestris*, the cause of brown rot in the cabbage, it is probable that, like the latter, the hyacinth germ is able to live for a long time in the soil of infected fields.

Diseased bulbs should be burned or put into a jar of dilute crude sulphuric acid, to which more acid is added from time to time. They should never be thrown into the canals or on waste land, nor should they be allowed to rot in place, for in this way all the soil would finally become infected. Land on which the disease is present should be used for other plants.

As suggested by Wakker, new varieties should be originated only by hand pollination, both parents being selected from such varieties as are naturally free from this disease, or which are at least little subject to it.

In concluding these remarks on pathogenesis it may be well to call special attention to certain features of this disease which seem especially instructive. The peculiarities which have impressed me most are: (1) The extremely slow progress of the symptoms—a slowness which is very remarkable if we compare it with the rapid action of such bacterial diseases as pear blight (*Bacillus amylovorus*) or the wet white rot of hyacinths which attacked some of my plants in 1898. (2) The extent to which the disease is restricted to the particular vascular bundles which are first invaded, i. e., the very slow invasion of the parenchyma and of remoter vascular bundles protected by this parenchyma.

This disease is not only peculiarly a vascular trouble, as Wakker pointed out, but is so restricted to the bundles first invaded that it seems to me impossible that there should ever be any general infection of the bulb scales until after the vessels which form a network in the plateau have become diseased. The disease was not observed in the roots.

The conditions under which this organism can grow parasitically appear to be narrowly restricted. It is not known to occur on any other host plant. It is a feeble, slow acting parasite and probably it would be confined to the domain of pure saprophytism were it not for the aeration and other peculiarly favorable conditions occurring in the vascular bundles of the hyacinth. The parenchyma of the bulb scales is distinctly acid and plainly unfavorable to its growth, most likely on account of this acidity, since studies of the organism in a variety of culture media have shown it to be peculiarly sensitive to the presence of acids, even those of the hyacinth (see Bulletin 28).

If the parenchymatic tissues of the hyacinth were less acid, if the germ were a more copious alkali producer, if it were less strictly aerobic, if it destroyed cell walls more readily, or finally, if it exerted a more powerful diastatic action on starch, it would, in my opinion, be a much more active parasite.

It is probable that a slight difference in the acidity of the parenchyma in different varieties of hyacinths is what renders some varieties more susceptible than others, but this can not be settled without further experiments which were best undertaken in the Netherlands, where according to Wakker the growers have long recognized that there are susceptible and nonsusceptible varieties.

The reader will be better able to judge of the correctness of these conclusions after reading Bulletin No. 28 in which the cultural peculiarities of this organism are discussed and compared with those of *Ps. campestris*, *Ps. phaseoli*, and *Ps. stewarti*, three other 1-flagellate, yellow bacteria common in the United States.

MORPHOLOGY OF THE PARASITE.

SIZE AND SHAPE.

This organism is a medium-sized slender rod, multiplying by fission. The ends are rounded. It is slightly variable in breadth and, under certain circumstances, greatly variable in length. Indeed, according to varying external conditions the length may be said to fluctuate enormously. Many examinations and measurements have been made. In the plant and in exhausted culture media it is generally only a single rod 1½ to 2 times as long as broad; rarely more than twice as long as broad. The appearance of some of the rods, which were taken from the daughter bulb examined in February, 1898, is shown in Plate I, fig. 8*a*. On slides stained 5 minutes in a saturated watery solution of basic fuchsin they were 0.4 to 0.5 by 0.5 to 1.0 μ. From the interior of a bulb of the first series (the slide stained June 23, 1897, in watery solution of basic fuchsin and mounted in Canada balsam and measurements made August 3, 1898), they were 0.5 by 0.9 to 1.5 μ, most of the rods on this slide being 0.5 by 1.0 to 1.2 μ. Taken from fresh cultures in beef broth they are a little longer. Plate I, fig. 8*b* shows typical forms from an alkaline beef-broth culture 9 days old. The thickest rods observed on slides made from this culture and stained in a saturated watery solution of basic fuchsin were 0.6 μ. Most of them measured 0.4 by 1.0 to 2.0 μ. On slides made the third day from a well clouded 1,000-cc. flask of distilled water containing 20 cc. of beef broth, and stained with Dr. V. A. Moore's modification of Loeffler's flagella stain, they were 0.5 to 0.7 by 1.0 to 2.0 μ. On slides made from slant agar cultures 5 days old (stock 207, acidity +22 of Fuller's scale), and stained with Alfred Fischer's flagella stain, the largest rods were 0.8 to 1.0 by 2.0 to 3.0 μ. Some of these flagella-bearing rods are shown in Plate I, figs. 9*a* and 9*b*. Flagella stains seemed to slightly increase the thickness of the rods or to render visible an outer part not stained by ordinary methods. In general the elements of this species appeared to me slenderer than those of *Ps. campestris*. Under the same conditions *Ps. phaseoli* is also a little plumper and shorter. Flagella-bearing rods of the former are shown in Plate I, fig. 10, and of the latter in Plate I, fig. 11.

Rods were frequently seen in process of division, and occasionally two pairs were found joined end to end. Chains were never seen in the host plant or in young cultures. Even in old cultures in beef broth (rim excluded) and on potato and standard nutrient agar free from sugar, they were very rare. Prolonged search would, however, sometimes be rewarded by the discovery of a chain of 6 to 12 segments. As in case of *Ps. campestris* the tendency to form chains in the ordinary culture media is very slight. In sugar agar, on the contrary, and also on banana, sweet potato, etc., chains and long rods are very common. These are usually mixed in with zooglœæ and the short elements. In such media the short elements often grow out into undivided filaments 50 to 150 μ in length. In many of these I was unable to discover even a trace of septa. In others the segments were distinct. Transferred to alkaline beef broth or common agar the long rods and chains disappear and the ordinary form abounds. This growth in the form of chains and filaments was observed repeatedly in cultures abounding in sugar; in fact, it may be produced at will by inoculating this organism into agar rich in grape or cane sugar. Two of these long rods taken from a 30 per cent cane sugar agar are shown in Plate I, fig. 8*c*. *Ps. campestris* and *Ps. phaseoli* behave in the same way in the presence of an excess of sugar.

No branched forms have ever been seen. Like *Ps. campestris* some of the rods appear to be slightly curved, but the chains are not crooked or twisted, as in case of vibrios.

MOTILITY.

The organism is motile, at least in early stages of its growth, in a variety of media. These movements, which are tumbling and darting, are accomplished by means of one long polar flagellum. This flagellum was stained only after repeated trials. It must be very effectually mordanted. I finally succeeded with Van Ermengem's nitrate of silver method, with Fischer's stain, and with Dr. V. A. Moore's modification of Loeffler's stain. As a rule the flagella were only feebly stained. The appearance of this organ is shown in Plate I, fig. 9. Figures of the flagella of *Ps. campestris* and *Ps. phaseoli* are introduced for comparison. In some cases it seems as if the flagellum were given off slightly below the end of the rod, both in this species and in *Ps. campestris*, but of this I could not be entirely certain. Motility was observed in potato cultures 2 to 4 weeks old, but I was never able to see any in rods taken directly from the closely packed yellow masses inside the bundles of diseased bulbs. This material was examined very carefully in distilled water.

ZOOGLŒÆ.

Zooglœæ are usually developed in solid and fluid cultures after a few days, the time of appearance varying greatly with the nature of the medium. In general they appeared much sooner in acid fluids than in alkaline ones. In beef broth made very strongly alkaline to litmus (neutral to phenolphtalein) by means of caustic soda, they did not appear until the close of the second week. In acid beef broth (unneutralized) they were commonly visible to the naked eye a day or two after clouding. In one instance, however, they appeared in an alkaline gelatin culture the second day after inoculation and were very numerous the third day. This gelatin was strongly alkaline with caustic soda (neutral to phenolphtalein) and was in a fluid state (28° to 29° C.), i. e., in a condition where any substance unfavorable to growth could act on the organism most effectively. May it not be that the zooglœa stage is a protective state entered into by bacteria whenever the physical or chemical conditions of the substratum are unfavorable to growth, these conditions being either independent of the organism, as in this case, or brought about by its own metabolism?

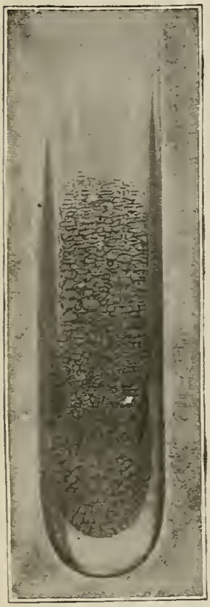

FIG. 3.—Culture of *Pseudomonas hyacinthi* on slant 30 per cent cane-sugar agar, showing "shagreen" surface.

In beef broth and other fluid cultures the tiny aggregations of this organism showed a marked tendency to gather into a ring or rim on the wall of the tube at the level of the liquid, and sometimes floating islands appeared, but the flocculent matter seldom united into any tough pellicle, being easily jarred apart and into the depths of the fluid. These zooglœæ appear to the naked eye either as small whitish flecks or, when on the rim at the surface of the liquid, as round, yellow, colony-like bodies, especially when they have reached some age and density. These bodies also formed on substrata rich in assimilable sugars; here, perhaps, owing to the development of acids. On the solid, sugar-rich substrata, e. g., sugar-agar, potato with sugar, sugar beet, sweet potato, etc., they produced a papillose, verrucose, or shagreen-like surface, the tiny rounded elements forming this surface being very smooth and distinct in their upper part, but fused below next to the substratum. This shagreen also appeared, on old cultures, on nutrient starch jelly containing 5 per cent glycerol. This appearance is shown in figs. 3 and 4.

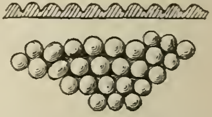

FIG. 4.—Slightly magnified diagrammatic views of slime of *Ps. hyacinthi* on sweet potato, showing "shagreen" surface.

SPORE FORMATION.

No spores have been seen, and I am in considerable doubt as to whether the spores observed in some of his cultures and studied so carefully by Dr. Wakker belonged to this species. He was never able to find any in the host plant, and those which appeared in his tubes may have been due to the fact that he was working at times with contaminated cultures. None of his successful infections with sporiferous material were made with spore masses entirely free from vegetative rods, and the latter are long-lived. This supposition of mixed cultures is the more likely because his work was done at a time when it was impossible to decide with ease and certainty on the purity of any given culture—i. e., before the era of poured plates—and especially because some of his gelatin cultures were certainly contaminated, i. e., yielded gas bubbles. (See Fermentation tube experiments described in Bulletin No. 28 dealing with the cultural characters of this organism.) While, therefore, not wishing to deny absolutely the existence of spores in this species, it seems to me that further and more exact proof is necessary to demonstrate their occurrence. A great many old cultures, grown on a variety of media at 18° to 26° C., have been examined without finding any spores. None were observed in the diseased bulbs, many of which were examined with care. Neither did any spores form in cultures exposed for fifteen days to air deprived of its oxygen by the potash-pyrogallic-acid method (test by microscopic examination and by exposure for ten minutes to 60° to 70° C. in alkaline beef broth). None developed in solid or fluid cultures exposed six weeks in the thermostat at 34° to 35° C. These cultures included alkaline and acid beef broth and cylinders of turnip and sugar beet standing in distilled water. Furthermore, this germ will not grow at all or grows only very feebly at the temperature which Dr. Wakker states to be most suitable for the formation of the spores viz. 35° C. (See Maximum temperature for growth, in Bulletin No. 28.) Finally, no spores developed in cultures which were first grown for a week or two at room temperatures and then put into the thermostat at 34° to 35° C. Several different media were tried, but vigorous growth stopped immediately, and after two weeks all such cultures were dead.

INVOLUTION FORMS.

Some astonishing involution forms have been observed. They formed a whitish rim at the surface of the fluid in strongly (soda) alkaline beef-broth cultures to which 10 per cent cane sugar had been added. The color was so pale that at first the tubes were supposed to be contaminated. When examined microscopically the cultures were five weeks old. These bodies were so immensely swollen, fused, twisted, and irregular in outline that seen on the slide no one to whom I showed them had any suspicion that they were bacteria. Involution forms were also seen on old turnip and banana cultures.

BEHAVIOR TOWARD STAINS.

Beyond the fact that the flagellum was stained with difficulty and that old growths, whether in the plant or out of it, took stains feebly, nothing peculiar was observed, unless it be that the bacterial precipitate resulting from growth was not stained in Dunham's solution containing methylene blue, and was stained in the same medium with rosolic acid. The following are transcripts from records scattered through my notes:

Germs from an old culture in strongly alkaline (soda) beef broth stained slowly and rather feebly in a saturated alcoholic solution of gentian violet diluted with an equal bulk of distilled water and allowed to act for half an hour. This culture had been killed by heat in the thermostat. Germs from an old culture in acid beef broth which had become alkaline, stained feebly in Ziehl's carbol fuchsin with ten minutes' exposure. This, also, was undoubtedly a dead culture. Germs from a month-old culture on sugar beet were exposed for some time to a dilute watery solution of gentian violet, whereupon all the zooglœæ stained deeply, but the loose rods rather feebly. On long exposure (over an hour) everything stained deeply. Germs from sweet potato cultures a month old (zooglœæ, rods, doublets, and chains) stained feebly in a deep-colored watery solution of gentian violet, although exposed for one-half hour. Germs taken from one of the bright-yellow bundles of a diseased bulb (June 23, 1897) stained feebly in water made deep red with Grübler's basic fuchsin. Germs from the yellow bundles of another bulb (Feb. 3, 1898) showed a very weak stain after five minutes' exposure to water saturated with Grübler's basic fuchsin. Exposed two minutes to water saturated with gentian violet, the stain was much better, but not deep enough. The rods from young cultures stain readily.

SYNOPSIS OF CHARACTERS.

For convenient reference I have drawn up the following brief account of this organism:

Pseudomonas hyacinthi (Wakker). A yellow, rod-shaped organism, multiplying by fission; ends rounded; single, in pairs, or 4's, more rarely in the form of chains or filaments; motile by means of one polar flagellum. In the host plant, when the bundles are crowded full of the yellow slime and broken down, it is, generally, 0.8 to 1.2 by 0.4 to 0.6 μ. In alkaline beef broth or on agar it usually measures 1.0 to 2.0 by 0.4 to 0.6 μ. In old cultures rich in sugar it often grows out into long, slender chains, or into filaments (50 to 100 μ long) in which there are no distinct septa. Nonsporiferous. Color distinctly yellow, but somewhat variable. Chrome yellow to pale cadmium in the host plant, i. e., bright yellow[1] (Ridgway's Nomenclature of Colors). On culture media, when not interfered with by the brown pigment, generally gamboge, chrome yellow, or canary yellow, but sometimes paler. Old cultures on some media darken from the production of a soluble, pale-brown pigment. This feeble brown stain is best developed in hyacinth broth, in potato broth with peptone, on turnips, on radishes, and on banana rinds. It was not observed in acid or alkaline beef broth, on coconut flesh, on sugar beets, in nutrient starch jelly, in agar, or in gelatin, with or without sugar. This organism grows readily on potato cylinders standing in distilled water, but it never becomes copious or fills the water with a solid yellow slime, owing to its feeble diastatic action. Potatoes on which it has grown, even for several months, always give a strong starch reaction with iodine. It behaves the same on nutrient starch jelly free from assimilable sugars. It liquefies nutrient gelatin and Loeffler's blood serum, but does so slowly, and will not liquefy gelatin at all if 10 per cent cane sugar is added (fig. 6). Growth on nutrient agar or nutrient starch jelly is inhibited (unless the inoculation be from a solid culture and very copious) by the addition of 10 per cent glycerol, and is greatly retarded by 5 per cent glycerol; even 2½ per cent of glycerol retarded growth. Growth in beef broth was much retarded by the addition of 1.5 per cent sodium chloride. Organism extremely sensitive to plant acids, including those of the hyacinth. Aerobic; doubtfully, if ever, facultative anaerobic; not a gas producer (see fig. 5). Does not redden litmus milk, but makes it bluer, and slowly separates the casein from the whey by means of a lab ferment. Produces under some circumstances, and slowly, a small amount of nonvolatile acid (slime acid?) with various sugars (grape, cane, etc.), which acid is frequently obscured by the moderate production of alkali. In the presence of air produces an organic acid (probably acetic) from ethyl alcohol dissolved in milk or bouillon. Inverts cane sugar, but apparently without the intervention of any enzym. Will not grow on 30 per cent grape-sugar agar. Resists dry air very well, i. e., more than forty-eight days when spread on cover glasses in thin layers.

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[1] Saccardo's *flavus* and *citrinus*, but brighter (Chromotaxia). The Standard Dictionary's *yellow III, lemon*, and *canary*, approximately (under *Spectrum*). Prang's *yellow*, Plate I y, in the Prang Standard of Color, Popular Ed., No. 1.

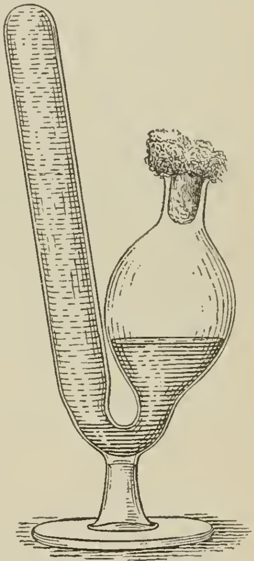


FIG. 5.—Typical behavior of *Ps. hyacinthi* in fermentation tubes containing peptone water, or peptonized beef bouillon, with addition of various sugars and other carbohydrates. Fluid clear in closed end, clouded in U and open end.

In Dunham's solution with methylene blue the color is reduced in a few days, but reoxidizes quickly on shaking; final color (56 days) bright blue. In Dunham's solution with indigo carmine the color changes to a bright blue, which persists for a long time; final color yellowish. In Dunham's solution with rosolic acid and enough HCl to render the fluid yellowish, *Ps. hyacinthi* did not redden the fluid, but made it colorless, the bacterial precipitate becoming rosy or salmon-colored. Produces indol slowly in peptonized beef broth and in peptonized Uschinsky's solution; does not produce nitrites in these solutions. Does not reduce potassium nitrate to nitrite in peptonized beef bouillon. Not a strong-smelling germ. Not readily destroyed by its own decomposition products except in media containing alcohol.

Will not grow in the thermostat at 37° C., and grows very feebly on some media and not at all on others at 34° to 35° C. Optimum temperature 28° to 30° C., or thereabouts. Minimum temperature approximately 4° C. Thermal death point (10 minutes' exposure) 47.50° C.; nearly all the rods are killed at 47° and a great many at 46.50° C. Did not grow at room temperatures after 6 days exposure in alkaline beef broth in the thermostat at 35° to 36.35°. Does not grow well in Uschinsky's solution. Grows much better in Uschinsky's solution when peptone is added to it. Grows well with a bright yellow color on cylinders of steamed coconut flesh, standing with one end in distilled water.

Pathogenic to hyacinths. Enters the plant through wounds, through the blossoms, etc., and multiplies in the vascular system, filling the vessels, especially those of the bulb, with a bright yellow slime consisting of bacteria. The walls of the vessels are destroyed and extensive cavities are formed in the bundles. The parenchyma around the bundles is also involved, but only very slowly, the organism being a feeble destroyer of cell walls. The host plant is not rapidly destroyed, a year or more being necessary. The cells are first separated by solution of the middle lamella, but the wall itself seems to finally disappear. The cavities contain innumerable bacteria mingled with fragments of the dissolved bundles and of the surrounding parenchyma.

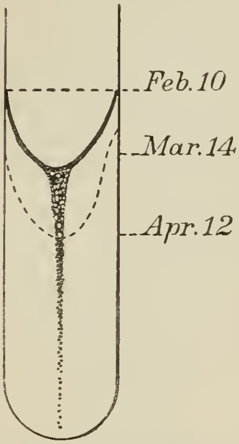


Fig. 6.—*Ps. hyacinthi* growing in strongly alkaline (0) gelatin with 10 per cent cane sugar. No liquefaction. The surface curves are due to the very gradual drying out of the gelatin.

First described by Dr. J. H. Wakker from the Netherlands, where it often causes serious losses in the hyacinth gardens. Not known to occur in any other part of the world.

REMARKS ON RELATIONSHIP.

Closely related to *Ps. campestris* (parasitic on Cruciferous plants), *Ps. phaseoli* (parasitic on beans), and less so to *Ps. stewarti* (parasitic (?) on corn, especially sweet corn). Readily distinguished from the two organisms first named by (1) its brighter color; (2) its lower thermal death point; (3) its manner of growth on potato cylinders standing in distilled water, i. e., by its feeble action on starch; and (4) its pathogenic properties. Other distinctions are given in Bulletin No. 28. Readily distinguished from *Ps. stewarti* by (1) its different, brighter color; (2) its feeble growth in Uschinsky's solution; (3) its liquefaction of gelatin and Loeffler's blood serum; (4) its lower thermal death point; (5) its lab ferment; (6) its much greater sensitiveness to acids; (7) its more luxuriant growth on turnip and rutabaga.

From facts in possession of the writer it is certain that there are many yellow organisms more or less closely related to the four mentioned in this paper, i. e., nonsporiferous, rod-shaped, micro-organisms, multiplying by fission, possessing one polar flagellum, and capable of living parasitically or semiparasitically upon various plants. All of these parasitic yellow organisms, at least all I have examined, are morphologically quite different from *Bacillus coli*, *Bacillus amylovorus*, *Bacillus tracheiphilus*, or any other micro-organism having flagella distributed over its whole surface. They also differ in many cultural peculiarities. They are, however, related to each other in many ways, and appear to form a natural group. I have an idea also that in some species the production of the brown pigment, and in others the production of the yellow pigment, has been nearly or quite extinguished. The species in which both pigments come the nearest to being equally well developed is, perhaps, *Ps. campestris*. The yellow pigment appears to be a lipochrome. (See Bul. 28.)

There are also, I believe, many morphologically similar yellow bacteria which are purely saprophytic.

EXPLANATION OF ILLUSTRATIONS.

TEXT FIGURES.

Fig. 1. Portion of a bulb scale from plant No. 20, inoculated February 7, drawn June 14, showing four healthy and four diseased vascular bundles. The parenchyma between two of the latter has largely disappeared, its place being occupied by a cavity full of bacteria. Smaller cavities in the parenchyma, close to the vascular tissue, are visible in each one of the diseased bundles. The bundle in the middle of the scale also shows the bacterial occupation of anastomosing veinlets. The diseased portions were bright yellow from the presence of enormous numbers of the parasite, which, however, had not reached the surface of the scale. The infection of this scale was from below upward. (Page 22.)

Fig. 2. Leaf of plant No. 25, inoculated February 7, drawn April 30. The figure shows shriveled apex and dead central stripe, narrow border of yellow, and beyond this to either side healthy green tissue (white in the figure). In the yellow border on the right side are some dotted areas intended to represent water-soaked tissue; i. e., spots recently invaded apparently by a slow sidewise movement of the bacteria from the central stripe. (Page 23.)

Fig. 3. Slant, 30 per cent, cane-sugar agar showing the "shagreen" surface. Culture No. 9, June 30, 1898. Photographed August 2. (Page 38.)

Fig. 4. Enlarged diagrammatic vertical and horizontal view of a similar shagreen surface from a sweet-potato culture twenty days old. (Page 38.)

Fig. 5. Fermentation tube showing behavior of *Ps. hyacinthi* in peptone water or peptonized beef broth with various carbohydrates, e. g., grape sugar, fruit sugar, cane sugar, milk sugar, galactose, mannit, glycerin, ethyl alcohol, etc. Maltose is a possible exception, tubes with this sugar having finally clouded *very feebly* in the closed end. None yielded any gas. (Page 41.)

Fig. 6. Stab culture in 0 gelatin + 10 per cent cane sugar inoculated February 10. On March 14 there was a well-developed stab and a good surface growth, but no liquefaction, the curved surface being due to the drying out of the gelatin. On April 12 the gelatin had dried out as indicated by the dotted line, but there was no liquefaction. (Page 42.)

PLATE FIGURES.

Fig. 1. Cross section of the bulb of plant No. 8, inoculated in the upper part of the scape February 16, 1897. Photographed June 23, 1897. Six vascular bundles broken down and filled with the bright yellow bacterial slime.

Fig. 2. Onion leaf, inoculated January 29, 1898. Painted by F. A. Walpole, March 5. The yellow color of the leaf in the vicinity of the inoculations was due to the slow and long-continued growth of the organisms; i. e., it is the yellow color of the bacteria showing through.

Fig. 3. Leaf of plant No. 51 (series 9), inoculated near the apex (at x) on February 11. Painted by F. A. Walpole, March 5. The water-soaked lines shown in the lower part of the stripe were conspicuous. This leaf was injected with 0.3 cc. of a cloudy beef-broth culture eight days old, but there were no symptoms until after the sixth day.

Fig. 4. Leaf of plant No. 52, inoculated at the same time, from the same culture, in the same manner, and with the same quantity of broth as No. 51 (fig. 3). Painted March 5 by F. A. Walpole. Symptoms farther advanced than in fig. 3, but none visible the first week.

Fig. 5. Cross section of the upper part of the bulb of plant No. 63, inoculated February 12, 1898, through the flowers. Photographed June 18. Eight scales visibly affected (16 vascular bundles). Farther down, near the junction of the scape with the plateau, a larger number of bundles and more scales were affected. The flattened side of this bulb (upper part of figure) is where a daughter bulb pressed against it (see text, page 29).

Fig. 6. One scale removed from the bulb of plant No. 63 (fig. 5) and photographed by itself, to show the course of the disease in the vascular bundles. The parenchymatic tissue between these yellow bundles was sound.

Fig. 7. Scape of plant No. 79, inoculated February 16 in the blossoms. Painted by F. A. Walpole, March 5. The infection proceeded apparently from one flower.

Fig. 8. (a) Ordinary form of bacterial rods found in the diseased bulbs. These were taken from the daughter bulb mentioned on page 18, and were stained two minutes in a saturated watery solution of gentian violet. × 1,000. (b) Ordinary form of rods from an alkaline beef-broth culture nine days old. Stained ten minutes in a saturated watery solution of Grübler's basic fuchsin. × 1,000. (c) Two long rods from a 30 per cent cane sugar agar thirty-eight days old. No segments or septa visible. Van Ermengem's nitrate of silver stain. × 1,000.

Fig. 9. (a) Flagella stained by Dr. V. A. Moore's modification of Lœffler's stain. Bacteria grown for three days in 1,000 cc. of distilled water with addition of 20 cc. of beef broth (see 18th series of inoculations). × 1,000. (b) Flagella stained by Dr. Alfred Fischer's stain. Bacteria from an agar culture five days old. × 1,000.

Fig. 10. Flagella of *Ps. campestris*, introduced for comparison. Bacteria from an agar culture seven days old. Fischer's flagella stain. × 1,000.

Fig. 11. Flagella of *Ps. phaseoli*, introduced for comparison. Bacteria from a culture twenty days old, on nutrient starch jelly with the addition of lactose. Van Ermengem's nitrate of silver stain. Ten minutes in the osmic acid mordant at 55° to 60° C. × 1,000.

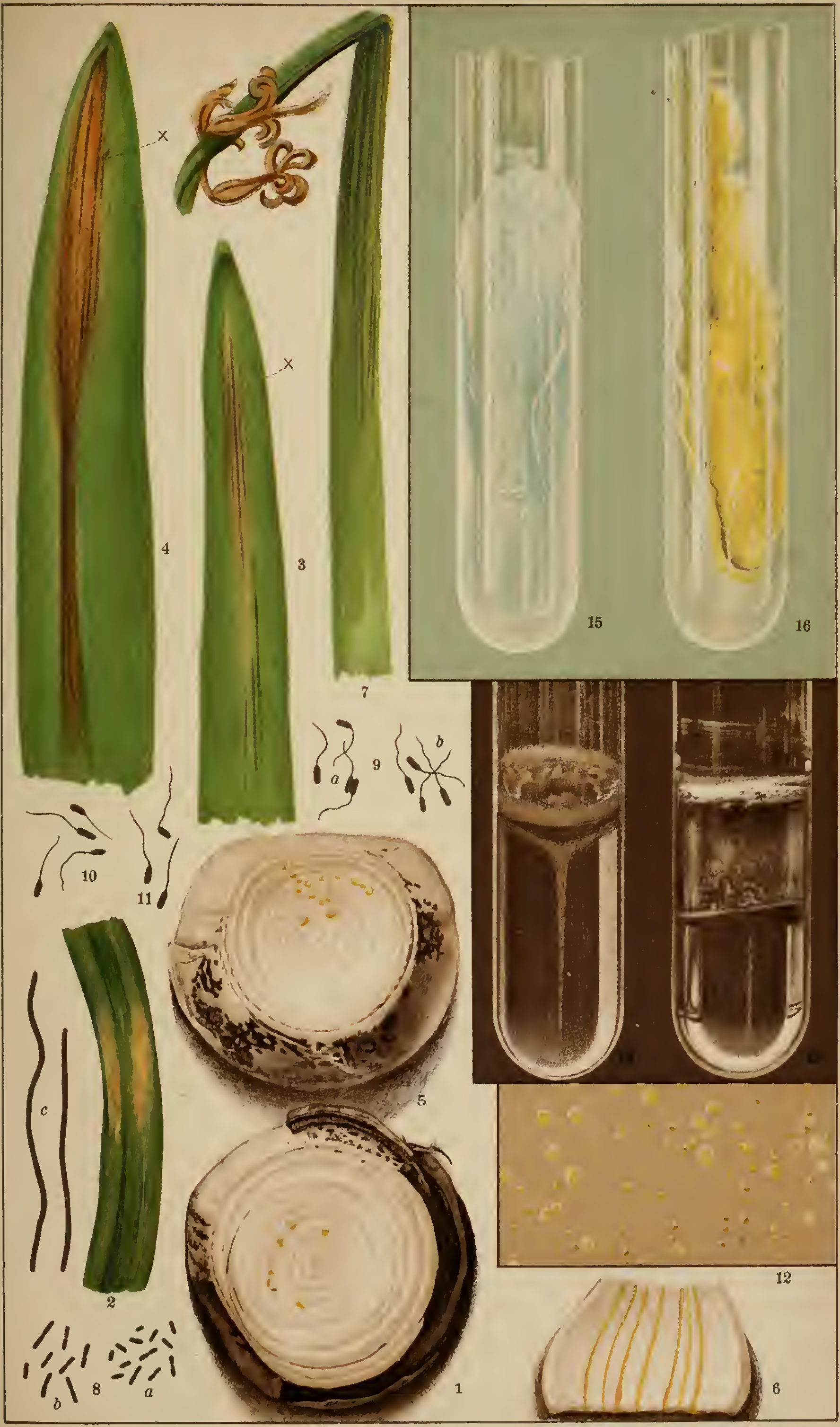
Fig. 12. Colonies of *Ps. hyacinthi* from a poured plate (Petri dish) of + 15.5 agar, after sixteen days at 22° to 23° C. The smaller colonies are buried ones. This plate was made from the 1,000 cc. flask culture (18th series of inoculations). The buried colonies are too deep a yellow in the lithograph.

Fig. 13. A stab culture in 8 per cent nutrient gelatin (+ 48 with malic acid) showing the very slow liquefaction. Photographed six weeks after inoculation. Range of temperature, 17° to 20° C. Upper one-half of the gelatin liquified to the walls, bright yellow precipitate and copious yellow rim. Lower one-half clear, solid, unstained, and showing in the center the whitish slender thread of the bacteria growing, very slowly, along the track of the needle nearly to the bottom of the tube.

Fig. 14. A stab culture in the same gelatin as 13, but with the addition of 5 per cent cane sugar. Photographed six weeks after inoculation. Range of temperature, 18° to 20° C. An equally good growth, but liquefaction entirely prevented by the addition of the cane sugar. Compare with *alkaline* sugar gelatin (text fig. 6.)

Fig. 15. A streak culture on nutrient starch jelly, fourteen days after inoculation. No visible growth, owing to absence of readily assimilable carbohydrate food. Painted by John L. Ridgway.

Fig. 16. A streak culture on nutrient starch jelly, fourteen days after inoculation. This culture was an exact duplicate of that shown in fig. 15, except that before the inoculation 20 milligrams of reprecipitated (sugar free) Taka-diastase was allowed to act on the starch one and one-half hours at 34° C., so that some of the starch was converted into readily assimilable substances. The diastase was then destroyed by steaming and the slant surface was inoculated in the same way as 15. Painted by John L. Ridgway.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ERWIN F. SMITH, F. A. WALPOLE
AND JOHN L. RIDGWAY.

A.HOEN & CO.LITHOCAUSTIC,BALTIMORE

PSEUDOMONAS HYACINTHI (WAKKER) ERW. SMITH.

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U. S. DEPARTMENT OF AGRICULTURE,
DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY.
ALBERT F. WOODS, CHIEF.

THE WILT DISEASE OF COTTON AND ITS CONTROL

BY

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ASSOCIATE PATHOLOGIST.

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U. S. DEPARTMENT OF AGRICULTURE,
DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY.
ALBERT F. WOODS, CHIEF.

THE WILT DISEASE OF COTTON AND ITS CONTROL.

BY

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Division of Vegetable Physiology and Pathology,
Washington, D. C., October 30, 1900.

Sir: I respectfully transmit herewith a report by Mr. W. A. Orton, of this Division, describing briefly the progress made in the study of the Wilt Disease of Cotton, also known as "Frenching" and "Blight." This disease has for several years done serious injury in many parts of the cotton belt. The areas affected by it are annually increasing in size, and each year brings to the Department reports of outbreaks in localities hitherto supposed to be free from the malady. It is at present a serious menace to the cotton industry. The parasitic nature and life history of the fungus causing the disease have been thoroughly discussed in a former report by Dr. Erwin F. Smith, of this Division. The work of the Department on this disease is still in progress, but it is thought best to present a brief outline of the more important results obtained up to this time. It has been found that certain races are resistant to the malady, and results obtained in the experiments and by certain growers cooperating with the Department indicate that resistant strains can be obtained quite readily by selection. All other methods of fighting the disease have so far proved ineffective. Every effort will therefore be put forth to improve and develop these resistant strains.

I respectfully recommend that this report be published as Bulletin No. 27 of this Division.

Respectfully,

Albert F. Woods,
Chief of Division.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

Page.
Distribution ... 5
Extent of loss ... 5
Description of the disease ... 6
Cause of the disease ... 6
Natural infections ... 8
Artificial infections ... 8
The failure of soil fungicides ... 9
Preventive measures ... 10
  Hygienic treatment ... 10
  Selection of resistant races ... 11
Control of other wilt diseases by selection ... 14
Conclusions ... 15
Explanation of plates ... 16

ILLUSTRATIONS.

Page.

PLATE I. Fig. 1.—Wilt disease in upland cotton, Dillon, S. C. Fig. 2.—Healthy field of upland cotton, Dillon, S. C. 16

II. Fig. 1.—Jannovitch, an Egyptian cotton, on the left; King, an upland cotton, on the right, showing comparative resistance to the wilt disease. Fig. 2.—Jackson on the left, Drake on the right, showing comparative resistance to the wilt disease 16

III. Fig. 1.—Root tufts produced on Jannovitch cotton by repeated partial infections by the wilt fungus. Fig. 2.—Egyptian cotton plants from infected and noninfected soil, showing dwarfing effect of the wilt fungus 16

IV. Sea island cotton resistant to the wilt disease. The result of selection from resistant plants 16

WILT DISEASE OF COTTON AND ITS CONTROL.

DISTRIBUTION.

The wilt disease is now known to occur on the coast of South Carolina, where it attacks the fine sea island cotton, and at Dillon, Salters, and other places in the same State, where it attacks upland cotton.

Prof. F. S. Earle, of the State experiment station, reports it to be widely distributed in Alabama, particularly in the southern part, and states that it is undoubtedly growing worse from year to year. It has been reported from many localities in Georgia, and is known to occur in Florida and Arkansas.

It is certain that this disease is widely distributed through the Southern States, and it is probable that it occurs in many places where it has not yet been distinguished from other troubles, such as "rust" and the effects of lightning.

EXTENT OF LOSS.

The annual loss from the wilt disease is very considerable. It is more keenly felt by the individual planters than most cotton troubles, because the disease remains in the soil and grows worse with each succeeding crop. On the sea islands of South Carolina alone a careful estimate indicates that nearly, if not quite, one-third of the land planted to high-grade cotton is affected by this disease, the larger portion of it so badly that it is no longer profitable to plant it in cotton. In many instances it has been necessary to abandon from 20 to 50 acres on a single plantation. Much of this land is tile-drained and in a high state of cultivation. No other crop has been so profitable as the sea island cotton, and the problem before these planters is a very serious one. The loss to the planters of upland cotton in areas affected by the disease has been proportionally great. On one farm in Dillon, S. C., where the Department has been conducting some experiments, 15 acres of fine land are already affected and the disease is spreading rapidly on this and adjoining plantations. The result of planting these infected soils with the ordinary varieties of cotton is shown in Plate I, in which fig. 1 shows a field of diseased cotton and fig. 2 a field of healthy cotton. The loss to this community from the wilt disease the past season is estimated at several thousand dollars. In Alabama the loss from this disease is reported from many sources to be very large.

The importance of the disease, however, does not lie so much in the amount of the present loss as in the danger of its future increase, for it must ultimately spread so much as to entail far greater losses and possibly threaten the life of the industry unless the methods for its control are perfected.

DESCRIPTION OF THE DISEASE.

The wilt is very distinct from any other disease of cotton, so that there need be no difficulty in its identification. It usually makes its first appearance in the spring about the last of May, when the plants are 6 to 8 inches high. It appears in well-defined areas, which enlarge if cotton is planted on the same land again. The first outward indication of its presence is a dwarfed growth and unhealthy appearance of the plants. The leaves turn yellow between the veins, their margins shrivel up, and some plants wilt and die at once. In other plants the progress of the disease is often slow, and many of them live the entire summer and die late in the season. On cutting across the stem of a diseased plant, the woody part will be found to be stained brown wherever the disease is present. In the absence of microscopic examinations, this brown discoloration of the internal tissue is the best ocular evidence of the presence of the wilt disease.

Plants may partially recover from a severe attack of the wilt disease by the development of strong lateral branches near the ground. Such plants may be distinguished by their dwarfed and bushy appearance and by the tendency of their branches to lie prostrate on the ground.

CAUSE OF THE DISEASE.

The cause of the wilt disease of cotton is a fungus, *Neocosmospora vasinfecta* (Atk.) Erw. Sm., which attacks the plant from the soil. It first enters the smaller roots and subsequently grows from these into the taproot and stem, filling the water ducts with its mycelium. The result is that the supply of food and moisture carried up from the roots is greatly decreased and the symptoms described above are produced. The nature of the fungus has been fully discussed in Bulletin No. 17 of this Division,[1] and it will not be necessary to enter into details here, but only to outline the subject and to record some additions to our knowledge.

The wilt disease of okra is believed to be caused by the same fungus which produces the cotton wilt. No inoculation experiments have been tried, but in the experience of the writer okra has never failed to contract the disease when planted in fields infected with the cotton-wilt disease.

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[1] Smith, Erwin F. Wilt Disease of Cotton, Watermelon, and Cowpea. 1899.

Both the wilt disease of okra and that of cotton are sometimes complicated by the presence in the field of the root nematode, *Heterodera radicicola*. The combined attack of these two parasites results in somewhat greater injury to the plants than would be caused by either one alone. This is particularly true of okra, which suffers considerably more from the attacks of the nematodes than cotton growing beside it. It is not believed by the writer, however, that the assistance of the root nematodes or of any fungus is necessary to allow the wilt fungus to gain entrance to the roots of cotton. Some of the worst cases of wilt disease that have been observed were on land where no root nematodes could be found. Nor is it believed that mechanical injury to the roots from cultivation or other causes is necessary to produce infection. Cotton planted on infected fields late in the season, after cultivation had ceased, and when conditions were not favorable to the growth of damping-off fungi in the soil, contracted the disease at the usual time. The indications are that the fungus is a sufficiently aggressive parasite to make its way unaided into the vascular system of the plant whenever the plant is liable to infection.

The progress of the disease is always slow as compared with that of other plant diseases. The period of incubation, or the time elapsing after the young seedling is exposed to the attacks of the fungus and before the disease becomes manifest, is usually at least forty days and often much longer. Much depends on the individual plant itself. The conditions which favor the progress of the fungus through the plant are not fully understood, but from some observations that have been made it is believed that highly fertilized plants, growing vigorously, succumb more readily than those which have grown on poorer soil.

In the early history of the wilt disease the cause was supposed by the planters to be the excessive applications or injudicious use of commercial fertilizers, and many of the leading planters in the Sea Islands made careful experiments with various modifications of their fertilizers, such as the use of marl, salt mud, kainit, and lime, and the increase or decrease of the proportions of phosphoric acid and potash. Mr. W. G. Hinson, of James Island, South Carolina, a very successful planter, has informed the writer that the result of all these trials has been to convince those who made them that the disease can not be controlled by any changes in their system of fertilizing.

The wilt disease occurs in so many widely separated localities and under such varied cultural conditions that it is not probable that any errors in the agricultural practice are the primary cause of the trouble, although the planting of cotton year after year on the same land and the common practice of plowing under the last year's stems in preparing the ground in the spring both tend to hasten the spread of the wilt fungus after it has once been introduced.

NATURAL INFECTIONS.

The effect of repeated infections of the small roots of the cotton is very noticeable, especially when the plants are somewhat resistant to the disease. Small tufts of roots grow from each point of infection, doubtless on account of some stimulus exerted by the fungus. Several short roots will thus start from a place which would normally have produced one longer branch. (Pl. III, fig. 1.)

Many of these little roots are killed by the fungus and others grow in their places, so that the tufted appearance of the rootlets is more pronounced late in the season. The same result has been produced in the laboratory by inoculating seedling cotton plants with pure cultures of the cotton-wilt fungus. Similar root tufts are found associated with the wilt diseases of okra, cowpea, watermelon, and cabbage, and they are believed to be characteristic of this class of root diseases.

In the case of cotton their presence on the roots demonstrates the presence of the wilt fungus in the soil, even when the amount is so small that no harm is visible aside from the reduced growth of the plants. (Pl. III, fig. 2.) This dwarfing of the plants is due to the killing of the small roots and is often visible over a considerable area surrounding a badly infected spot. For this reason the loss in yield on such a field is much greater than would appear simply from a consideration of the badly diseased areas, as the dwarfing due to the injuring of the small roots considerably curtails the yield.

ARTIFICIAL INFECTIONS.

Since the publication of Bulletin No. 17, the wilt disease has been produced in healthy cotton plants by inoculating the soil in which they grew with pure cultures of conidial stages of *Neocosmospora vasinfecta*. This removes any doubt as to the causal relation of the fungus to the disease which might arise from the failure of the previous inoculation experiments. The plants were grown for a few weeks in pots, and then a small quantity of fungus from a pure culture was placed in the bottom of each one. Eight days later they were transplanted to the open ground. The first case appeared after about 35 days. Fourteen out of 24 plants contracted the disease. The fungus was abundant in the vascular bundles of 7 plants and they showed all the other symptoms of the disease. The other 7 infected plants were only slightly diseased, although the fungus was found in the vessels of the stem. The check plants, 25 in number, all remained healthy. It will be noted that the length of time between the inoculation of the soil and the appearance of the disease in this experiment (35 to 50 days) was practically the same as elapses in the field between the germination of the seed and the first appearance of the disease. That a larger proportion of the inoculations did not succeed is believed to be due to the small amount of fungus used and to the natural resistance of the plants. The cotton plant inoculations described in Bulletin 17 were all made in the greenhouse and it is now believed that the negative results were due either to the slow growth of the plants or to the fact that they were naturally resistant.

THE FAILURE OF SOIL FUNGICIDES.

Careful experiments have been made with a large number of substances applied to the soil in the hope of killing the fungus, but all the results obtained up to the present time indicate that there is no hope of success from the use of any fungicides sprayed on the plants or applied to the soil.

Fields uniformly infected with the wilt disease were selected, and over twenty different substances were applied in amounts as large as it was thought safe to use. In many cases the expense of their application in such quantities was so great as to make their use impracticable had they proved efficacious. In other cases, as in the use of materials containing copper, continued applications in such large quantities would be likely to injure the soil.

The following were among the fungicides tried, nearly all of which were tested in duplicate or triplicate (in different localities):

1. *Bordeaux mixture*, 1,200 gallons per acre, applied to the soil ten days before planting.
2. *Bordeaux mixture*, 1,200 gallons per acre, applied to the soil before planting as above, and also sprayed on the plants and soil at intervals during the summer.
3. *Bordeaux mixture*, 1,200 gallons per acre, with the addition of a small quantity of molasses to increase the solubility of the copper.
4. *Bordeaux mixture and sulphur*, prepared by adding to each barrel of a mixture containing the ordinary amounts of copper sulphate and lime 6 pounds of sulphur and 6 pounds of lime that had been boiled together one hour. This was mixed with the soil in the row ten days before planting.
5. *Bordeaux mixture*, 3,600 gallons per acre. This is equivalent to 546 pounds of copper sulphate per acre, but the cotton grew well here until attacked by the wilt disease. To all appearances neither the cotton nor the wilt fungus was affected by this very heavy application, which was on a rather small plot (250 square feet).
6. *Copper carbonate*, applied in solution to the soil just before planting. at the rate of 136 pounds per acre.
7. *Copper acetate*, applied in solution to the soil just before planting. at the rate of 102 pounds per acre.
8. *Lime* (fresh stone lime) was applied to infected land in September, 1899, at the rate of 3, 4, 5, and 6 tons per acre. The lime was harrowed in as soon as it had become slacked. An equal area was left untreated, and cotton was planted in the usual way in 1900. Lime was also applied to other infected fields in the spring, shortly before planting. at the rate of 2.000 and 4.000 pounds per acre.
9. *Sulphur* (flowers of sulphur) was applied to the soil before planting. at the rate of 400 and 600 pounds per acre.
10. *Lime-sulphur mixture*, consisting of 30 pounds of lime, 20 pounds of sulphur, and 60 gallons of water. The lime was slacked and boiled with the sulphur one hour. It was applied at the rate of 600 and 900 gallons per acre.

11. *Liver of sulphur*, applied in solution before planting, at the rate of 30 pounds per acre.

12. *Iron sulphate*, applied in solution before planting, at the rate of 100 pounds per acre.

13. *Carbolic acid*, applied at the rate of 12 and 18 gallons of crude acid per acre.

14. *Caustic soda*, applied in 8 per cent solution at the rate of 1,000 pounds of commercial caustic soda per acre.

15. *Formalin*, applied in 10 per cent solution at the rate of 100 pounds of the commercial (40 per cent) formalin per acre.

16. *Kainit*, used at the rate of 2,000 and 4,000 pounds per acre.

For the purpose of comparison, in all cases, untreated plots were left beside those subjected to the foregoing treatments. The wilt disease was very bad on these fields, and nearly all the cotton died on both the treated and the untreated plots, but no difference traceable to the fungicides used could be observed between them.

A test was also made of "Brown's Watermelon Wilt Remedy", which has been put on the market as a preventive for both the cotton and the watermelon diseases. This treatment consisted in soaking the seed twenty-four hours in a patent compound,[1] with the addition of a small amount of air-slacked lime to the soil before planting. It was given a careful trial, according to the directions of the maker, but no difference was observed between the treated plots and the untreated plots beside them.

At the request of the Department, the same remedy was tested on watermelons by Mr. T. S. Williams, of Monetta, S. C., a well-known grower of melons, who has had much experience with the watermelon wilt disease. The seed for 200 hills was treated according to directions, and 200 other hills beside them were left untreated. A perfect stand was obtained, and the plants were thinned out to one in a hill. A careful count sixty-five days after planting showed 195 of the 200 treated plants killed by the wilt disease. In the other (untreated) row 187 of the plants were killed by the fungus.

PREVENTIVE MEASURES.

HYGIENIC TREATMENT.

In the cotton wilt, as in many other plant diseases, certain preventive or palliative measures, based on our knowledge of the way the disease spreads, are very important. These are as follows:

(1) *Rotation of crops.*—Land once infected with this disease has never been freed from it. It is important, therefore, that such land should not be planted for several years to okra or any variety of cotton subject to this disease. The length of time the fungus will live in the soil is not yet determined, but four years' rest has proved insufficient in several cases. Other crops—as corn, cowpeas, cabbage, watermelon, etc.—may, it is believed, be planted on this land with safety.

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[1] Stated by the Division of Chemistry, to which samples were submitted, to be made of a mixture of liver of sulphur and lime.

The greatest spread of the wilt disease is by the direct growth of the fungus through the soil from diseased to healthy areas. On this account an area considerably larger than that on which the plants are wilting should be included in this rotation.

(2) *Removal of diseased plants.*—Another important source of infection is the diseased plants themselves. The fungus produces on the dead stems and roots great numbers of spores, which are carried to other places in a variety of ways. All diseased plants should be pulled and burned as soon as discovered, so as to prevent the dispersion of the fungus spores which will finally cover them.

(3) *Avoidance of spread by cattle, tools, etc.*—The writer's observations in various places in South Carolina during the past two years lead him to believe that cattle grazing in the fields spread the disease. They should not be allowed to pass freely from infected areas to healthy fields, and it would be better not to pasture such infected fields. Tools should be carefully cleaned after cultivating the diseased land. To insure complete destruction of the spores of the wilt fungus, such tools should be scoured clean and then washed with a 2 per cent solution of formalin or a 5 per cent carbolic acid solution.

(4) *Care of the compost heap.*—The fungus is sometimes introduced into the barnyard and compost heap, so that the manure becomes a source of general infection to healthy fields. The utmost care should be taken to keep diseased plants out of the manure, and if there are any indications that such plants have found their way into the manure, or if any new outbreaks of the disease are traced to the use of stable manure, all such manure and compost should be used on land where cotton will never be planted.

There is no objection to the use of stable manure which does not contain the spores of the cotton-wilt fungus, but experience has shown that in the case of the closely allied watermelon-wilt fungus a barnyard once infected will remain so for many years, and that all manure taken out of it will be likely to spread the disease. The same is probably true of the cotton-wilt fungus.

SELECTION OF RESISTANT RACES.

The most encouraging results have come from the endeavor of the Department to find a race of cotton which can be grown on the infected lands. There are always some plants in every field which resist the disease to a greater or less extent, and it frequently happens that of two plants in the same hill, equally exposed to infection, one will die and the other live to the end of the season. All degrees of resistance may be found, from plants nearly killed by the wilt disease to those entirely healthy. The latter are comparatively uncommon, however.

Different races of cotton vary considerably in their susceptibility to the wilt disease. This was shown by an experiment carried out by the Department on the farm of Mr. H. L. Galloway, at Dillon, S. C.

Twenty races, including the more prominent ones in cultivation, were planted in a field that was thoroughly infected with the wilt disease, and their comparative resistance determined in August by counting the number of plants remaining healthy, those partially diseased, and those killed.

None of the races tested were entirely resistant, but some showed great promise in this regard. The greatest resistance was shown by the Egyptian cottons, Mitafifi, Abbasi, and Jannovitch, which withstood the disease to a very marked extent. Very few plants were killed outright, although nearly all were considerably reduced in size and yield. The striking difference in resistance between these sorts and an ordinary upland cotton (King) is shown in Pl. II, fig. 1. The race figured here, the Jannovitch, was imported from Egypt by the Department through Messrs. Barbour Lathrop and D. G. Fairchild. It is a long-staple cotton of fine quality, said to be the result of a cross between the Egyptian and sea island cotton, and regarded as being adapted to upland culture. It has been widely distributed during the past year, and promises to be of great value. The other Egyptian sorts, Mitafifi and Abbasi, which were introduced at the same time as the Jannovitch, were also very resistant. They differed from that sort chiefly in the yield and the color of the lint. The most productive strain of Egyptian cotton grown on infected land was Mitafifi, No. 3992.

Sea island cotton, although closely related to the Egyptian, suffered very much. It was practically no more resistant than the upland cotton growing beside it. Nearly all the upland races proved very susceptible to the disease, though there were minor variations which must have been due to varietal differences.

One race only, the Jackson (Limbless), showed a marked resistance. In this respect it far surpassed all other upland cottons and nearly equaled the Egyptian. (See Pl. II, fig. 2.) The yield of this race on wilt-infected land was very good. Many plants were injured by the disease, but many others were exceptionally vigorous and there is no doubt that selection from these healthy plants would greatly increase the percentage of resistance. The relative resistance of these races in the experiment mentioned is shown in the following table:

Table showing varietal resistance of cottons to the wilt disease.

[The figures denote the comparative resistance of the different races on a scale of one thousand.]

Jannovitch	565
Mitafifi (average of 3 strains)	559
Abbasi	479
Jackson	453
Sea island	233
Eldorado	227
Texas Wood	162
Doughty	148
Hawkins Prolific	142
Brady	127
Cook's Long Staple	124
Excelsior	104
Drake	90
Jones	88
King	83
Peterkin	71
Truitt	71
Russell	55

It will be seen that some of the best kinds are among those most injured by this disease; but there were one or more plants in each race that entirely withstood the disease, and the seed from these has been saved with the intention of securing valuable resistant strains by cross-breeding them.

The ability of certain cotton plants to grow on infected land is due to the fact that the wilt fungus is unable to enter their principal root system and not to any lack of infection. This has been determined by microscopic examination. That infection of these plants has really taken place may be demonstrated by an examination of their roots for the little tufts of rootlets which mark the location of infections (see p. 8). The roots of plants taken from the row of Jannovitch cotton shown in Pl. II, fig. 1, were attacked by the fungus in over a hundred places, as found by actual count, yet in no case did the parasite penetrate as far as the main stem, while plants of King cotton in the adjoining row were completely overcome. A part of the root system of one of these resistant plants is represented in Pl. III, fig. 1. As determined by numerous microscopic examinations each little tuft of roots marks a point attacked by the fungus, so that there can be no doubt of the thoroughness of the infection and, furthermore, no doubt that such plants are actually resistant to the fungus.

It is evident that such an effect as the fungus has produced here must injure the plant considerably and this was found to be the case. The average height of plants grown on the infected land was 23 inches, while plants on adjoining land very slightly infected grew 42 inches high. Plate III, fig. 2, shows the difference between these plants. Such injury as this would of course greatly shorten the crop, but the indications are that seed selected from the most vigorous plants will be more resistant than the average. The best plants in our experimental plots on the infected land were nearly equal to those grown on healthy land and also showed a smaller number of root tufts.

In this connection the most important question is whether this quality of resistance to disease is transmissible through the seed to succeeding generations. An experiment designed to settle this point proved a remarkable success. It was carried out by Mr. Elias L. Rivers, of James Island, S. C., who selected a healthy plant of sea island cotton that grew in a badly blighted field in 1899. The seed from this resistant plant was saved and planted in a single row through a field that had been infected with the wilt disease for several years. The adjoining rows were planted with seed from his main crop, grown on noninfected land. The result is indicated in the photograph (Pl. IV) taken September, 1900. The wilt disease made almost a clean sweep through the ordinary cotton, 95 per cent of the plants being killed, while in the row planted with seed from the resistant plant *not a single plant was killed by the wilt disease.*

These plants were vigorous and productive. The dwarfing noted in

Egyptian and upland cotton grown by the writer on infected land at Dillon, S. C., was not so marked here. The quality of the lint was good, though not equal to the crop from which the selection was made. It is believed, however, that by continued cross breeding and selection in succeeding years the quality of the cotton may be improved without loss of resistance to the wilt disease. Work along this line has already been started in a small way by the Department, which it is hoped may be enlarged.

It has been shown that much can be accomplished in the control of the wilt disease of cotton by simply selecting seed from resistant plants. It is very probable that better results will be obtained by cross-breeding these resistant individuals, for in this way the resistant qualities of two plants will be combined and there will be added the increased vigor which usually comes from crossing. On the other hand, if the flowers of a resistant cotton plant should be fertilized by pollen brought by insects from a diseased plant, as may easily happen in the field, plants grown from the resulting seeds will very likely be less resistant than if they had been fertilized by pollen from another resistant plant. On this account, in the selection of resistant races, it will be desirable to cross by hand as many flowers as possible in order to increase the chances of success.

In connection with the work of the Department a large number of crosses between resistant plants have already been made. It has been our aim to secure resistant strains from our common races by cross-fertilizing plants of the same race, and at the same time to increase the productiveness and improve the quality by selecting the best plants of each sort for breeding.

The fact that the Egyptian cottons are resistant to the wilt disease has led to the attempt to produce a resistant long-staple upland cotton, by hybridizing resistant plants of the common upland races with the Egyptian cotton. It is very desirable that everyone who undertakes the breeding of resistant cotton should at the same time pay great attention to securing a more productive race and a finer quality of staple.

CONTROL OF OTHER WILT DISEASES BY SELECTION.

The indications are that other diseases similar to the cotton wilt may also be controlled by the selection of resistant races.

The wilt of the cowpea, which is a troublesome disease in many parts of the South, is caused by a fungus closely allied to that producing the cotton wilt (*Neocomospora vasinfecta* var. *tracheiphila*). In this case we already have a race, known as Little Iron, which will grow on infected land. A fine crop of this pea was grown during the past season by Mr. T. S. Williams, of Monetta, S. C., on fields where the whole crop was lost last year and where other races planted alongside it this year have been practically ruined.

Further investigations will probably result in the discovery of other races of cowpeas which may be so improved by selection that they may be planted on land infected by the wilt disease.

The wilt disease of watermelons, also allied to the two preceding, may prove amenable to the same treatment. The Department has under way some experiments to determine the possibility of finding a race of watermelon which may be grown on infected land. This would be exceedingly desirable, for this disease has made the growing of melons for market impossible over large areas in the South which formerly produced them in great abundance.

CONCLUSIONS.

There is great promise of a successful remedy for the cotton-wilt disease in selection of seeds from healthy plants growing on infected soils and by continuing to select and cross-breed the most resistant plants in succeeding crops with a view both to resistance and quality of staple.

It would be well in the case of upland cotton to start with a race like the Jackson, which is already highly resistant, and improve and fix the quality by careful cross breeding and selection. In places where this cluster type of cotton is undesirable a resistant strain of the sorts commonly cultivated can probably be obtained by cross breeding and selection. It is hardly to be expected that this process will result in a perfectly immune race the first year. Even though much of the cotton become diseased, the selection should be continued each succeeding year until the quality of resistance is fixed.

In the case of the sea island cotton, where length and fine quality of staple are essential, the process of selection and breeding should be the same. Resistance to disease must be the primary requisite, and from the resistant plants those bearing the finest lint may be selected.

The Egyptian cottons will probably prove of the greatest value when crossed with our upland races so as to add the vigor and quality of the former to the productiveness of the latter. It is hoped that the Department will be able to extend its work along this promising line.

In addition to selection for resistance, all practicable preventive measures should be applied. Rotation of crops is even more important on these infected soils than on healthy ones, for the continual growing of cotton on these lands will increase the amount of disease and decrease the resistance of the cotton.

Prompt destruction of diseased plants is also very important. Every effort should be made to avoid the infection of healthy fields by animals, tools, wash water from diseased fields, diseased plants, infected compost, etc. As already stated, land once infected with this disease remains infected for an unknown period.

EXPLANATION OF PLATES.

Plate I. Fig. 1.—A field of ordinary upland cotton at Dillon, S. C., showing the damage caused by the wilt disease (photographed in August, 1900). Fig. 2.—Field of healthy upland cotton adjoining the infected field shown in fig. 1 (photographed in August, 1900).

Plate II. Comparative resistance to wilt disease of different races of cotton in experimental plots of Division of Vegetable Physiology and Pathology, on the farm of Mr. H. L. Galloway, at Dillon, S. C. (photographed August, 1900): Fig. 1.—Jannovitch, an Egyptian cotton, on the left, and King, a common upland race, on the right. Fig. 2.—Jackson, on the left, and Drake, on the right. These plots were planted at the same time and treated exactly alike.

Plate III. Fig. 1.—Root tufts produced by partial infection of resistant plants. The roots figured here were from a plant of Jannovitch cotton taken from the row shown in Pl. II, fig. 1. There were about one hundred and fifty of these little tufts on this root. (Drawn by Mr. W. R. Scholl, from a root collected in September, 1900.) Fig. 2.—Egyptian cotton plants, showing the dwarfing effect of numerous partial infections of the small roots. The plant at the left came from noninfected soil and is healthy, while that at the right grew on infected land near by. (Drawn by Mr. W. R. Scholl, from a photograph.)

Plate IV. Sea island cotton resistant to the wilt disease (photographed September, 1900, on the plantation of Mr. Elias L. Rivers, James Island, S. C.). The row in the foreground was planted with seed from a single healthy plant that grew in infected land the year before. The adjoining rows, now almost entirely killed by the wilt disease, were planted with seed of the ordinary, fine sea island cotton. There are a few scattered plants in these rows that have resisted the disease. It was from such a plant as these that the seeds planted in the middle row were taken.

○

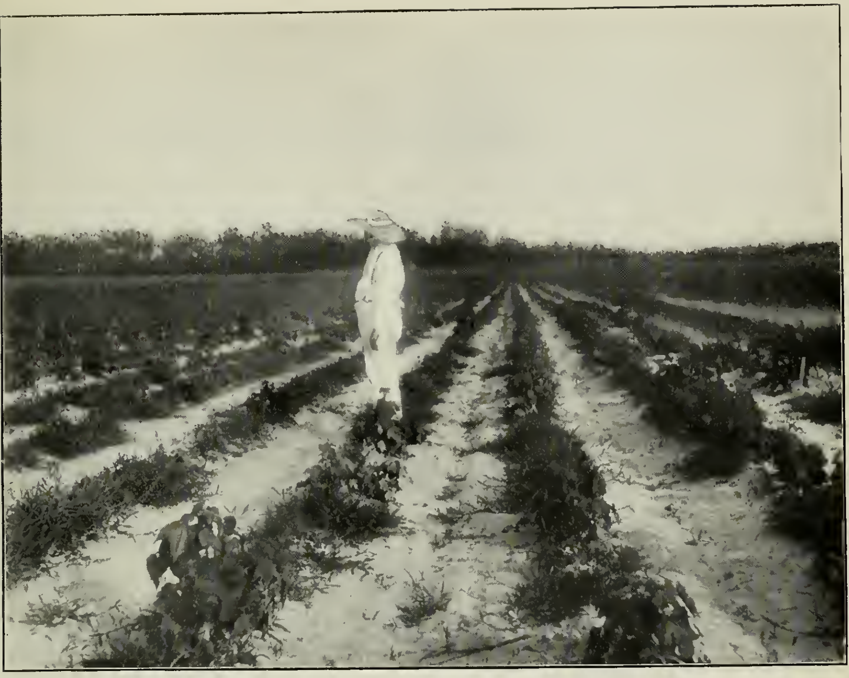


FIG. 1.—WILT DISEASE IN UPLAND COTTON DILLON, S. C.

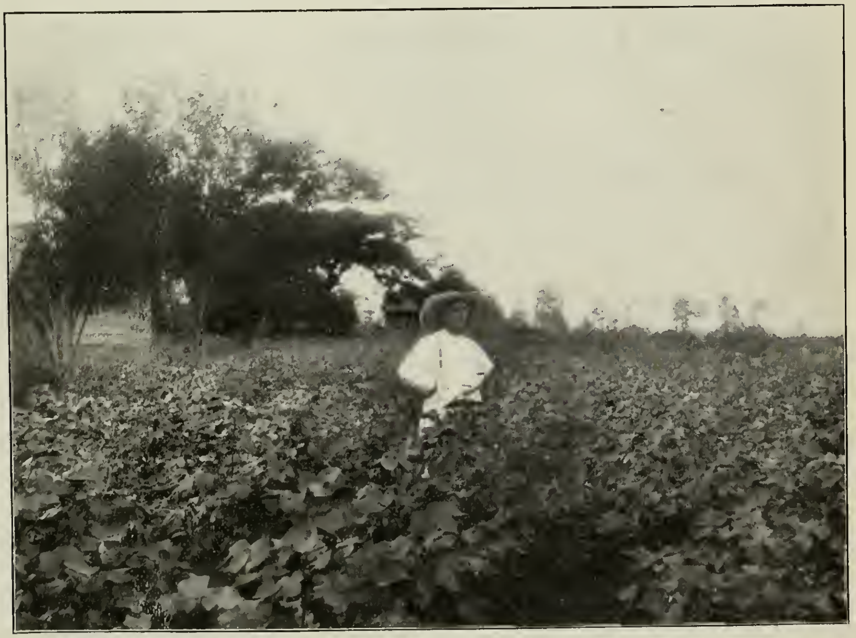


FIG. 2.—HEALTHY FIELD OF UPLAND COTTON, DILLON, S. C.

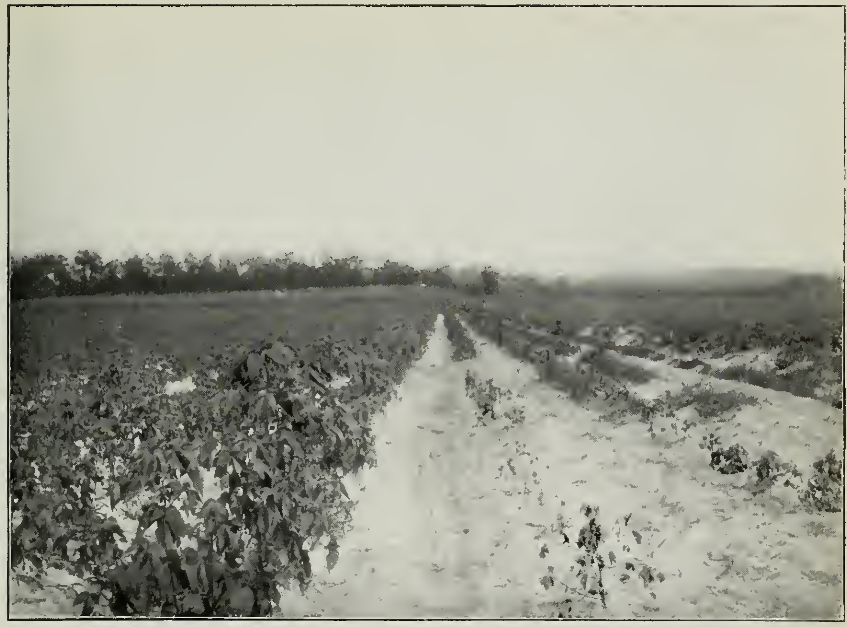


FIG. 1.—JANNOVITCH, AN EGYPTIAN COTTON, ON THE LEFT; KING, AN UPLAND COTTON, ON THE RIGHT, SHOWING COMPARATIVE RESISTANCE TO THE WILT DISEASE.

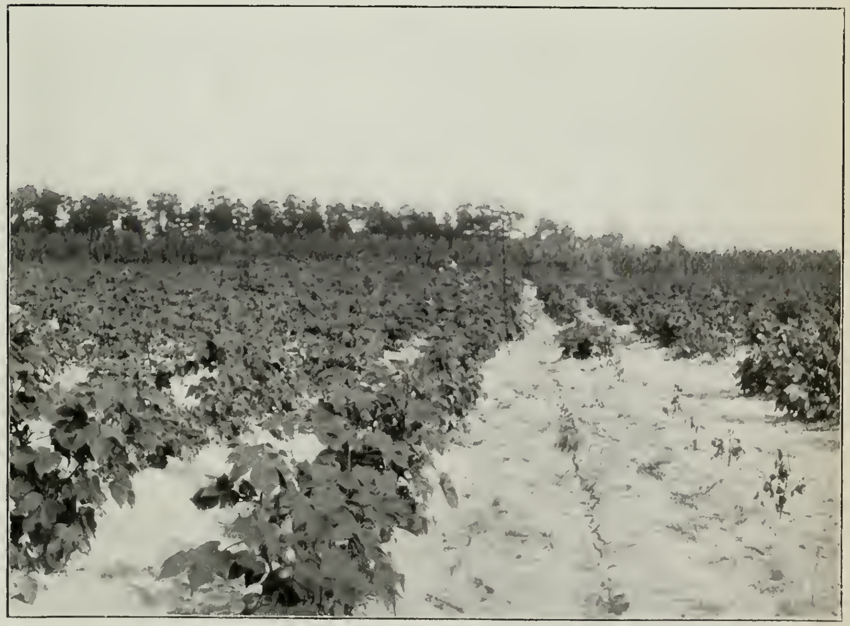


FIG. 2.—JACKSON ON THE LEFT, DRAKE ON THE RIGHT, SHOWING COMPARATIVE RESISTANCE TO THE WILT DISEASE.

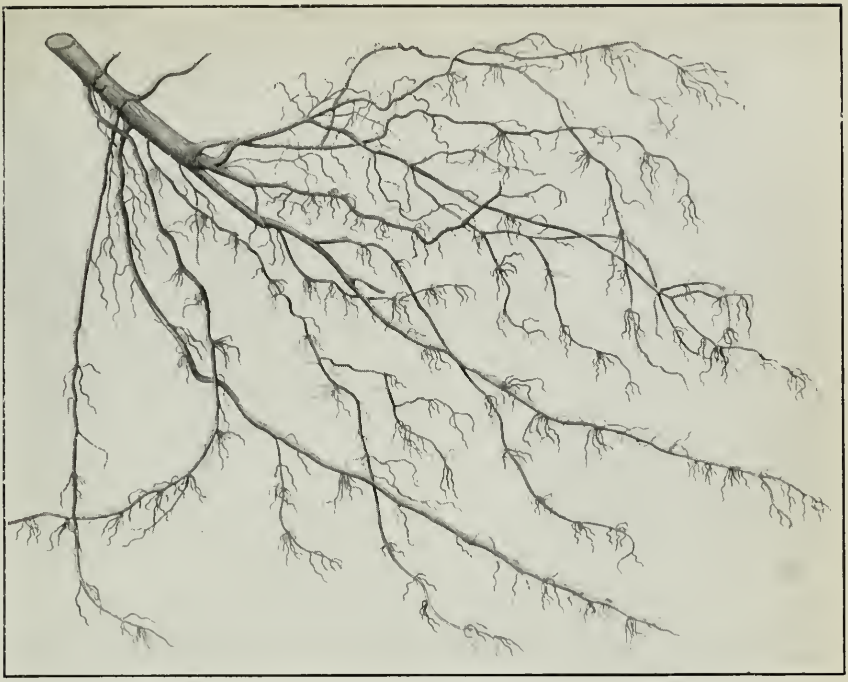


FIG. 1.—ROOT TUFTS PRODUCED ON JANNOVITCH COTTON BY REPEATED PARTIAL INFECTIONS BY THE WILT FUNGUS.

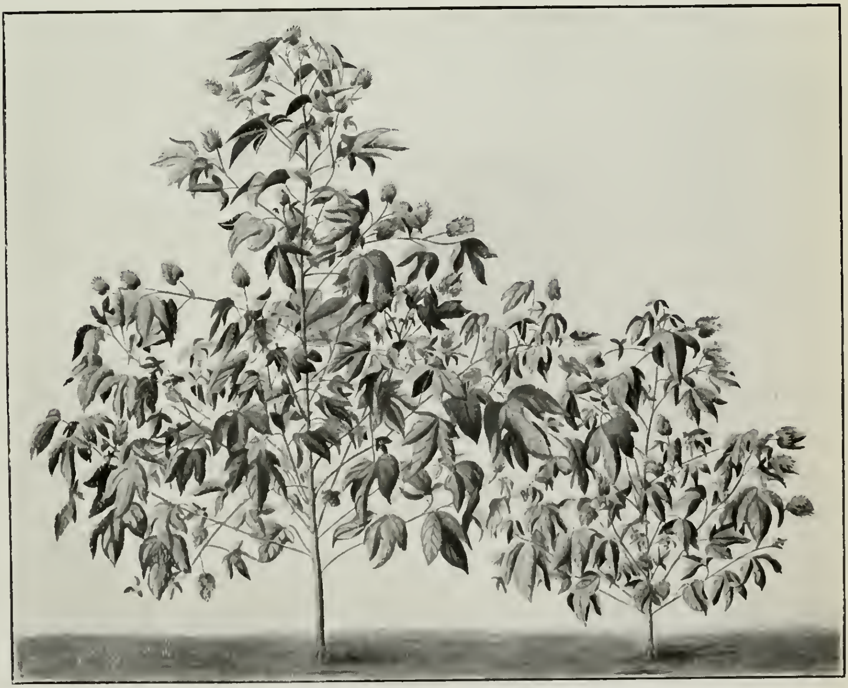
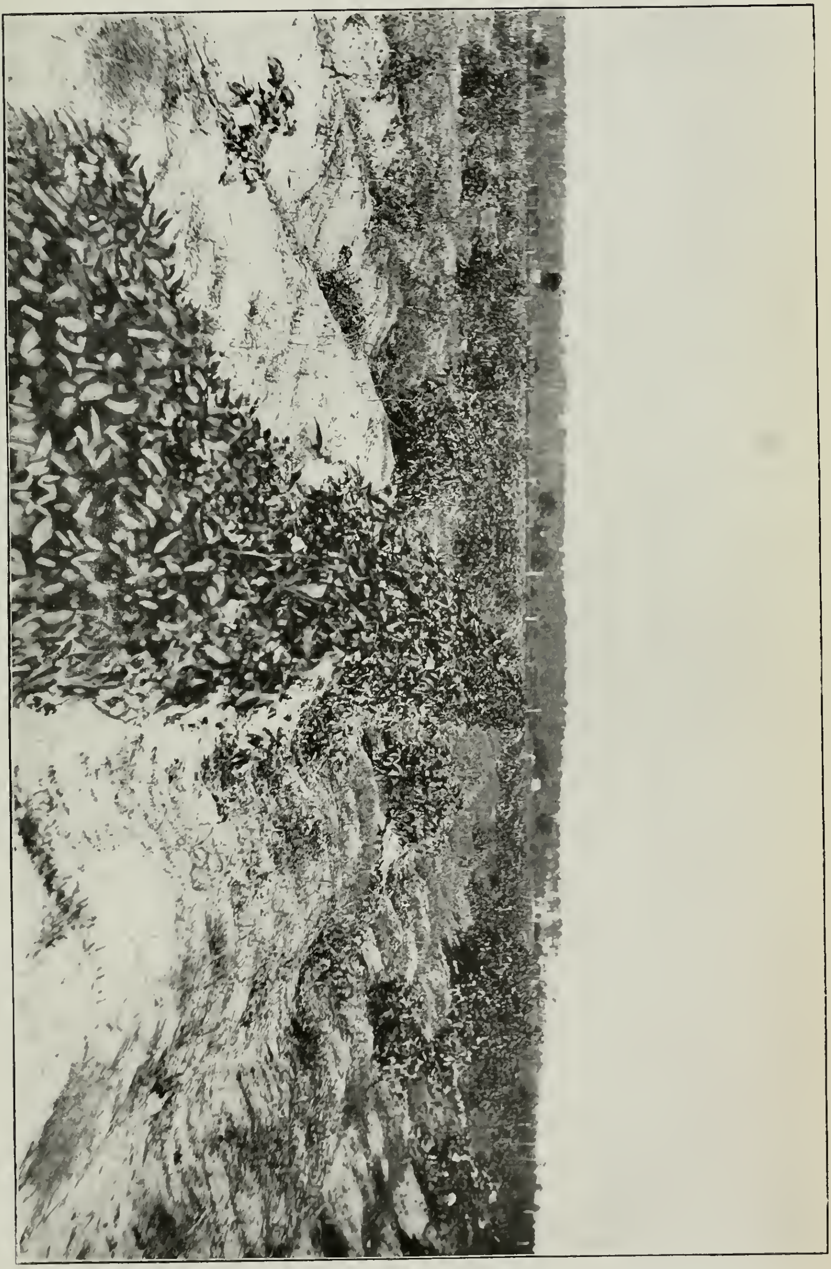


FIG. 2.—EGYPTIAN COTTON PLANTS FROM INFECTED AND NONINFECTED SOIL, SHOWING DWARFING EFFECT OF THE WILT FUNGUS.



SEA ISLAND COTTON RESISTANT TO THE WILT DISEASE. THE RESULT OF SELECTION FROM RESISTANT PLANTS.

BULLETIN NO. 28. V. P. P.—82.

U. S. DEPARTMENT OF AGRICULTURE,
DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY.
ALBERT F. WOODS, Chief.

THE CULTURAL CHARACTERS
OF
PSEUDOMONAS HYACINTHI, Ps. CAMPESTRIS, Ps. PHASEOLI, AND Ps. STEWARTI—FOUR ONE-FLAGELLATE YELLOW BACTERIA PARASITIC ON PLANTS.

BY

ERWIN F. SMITH,
Pathologist, in Charge of Laboratory of Plant Pathology.

ISSUED AUGUST 6, 1901.

NEW YO
BOTANI
GARDE

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1901.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Division of Vegetable Physiology and Pathology,
Washington, D. C., January 15, 1901.

Sir: I have the honor to transmit herewith the manuscript for a bulletin by Dr. Erwin F. Smith, of this Division, on the cultural characters of *Pseudomonas hyacinthi*, *Ps. campestris*, *Ps. phaseoli*, and *Ps. stewarti*—four one-flagellate yellow bacteria parasitic on plants. The first is the cause of a serious disease of hyacinths, described in Bulletin No. 26 of this Division; the second is the cause of a widely distributed and destructive disease of cabbages, known as brown rot and described from a practical standpoint in Farmers' Bulletin No. 68; the third is the cause of a serious disease of beans, and the fourth is believed to be the cause of a serious disease of sweet corn. The Bulletin also contains occasional references to *Bacillus amylovorus*, *B. coli* and other bacterial organisms which were used for comparison. It is the first exhaustive working over of an interesting group of plant parasites, concerning which practically nothing was known in 1896 when Dr. Smith began his studies. The work described is of a purely technical nature, but will be valuable to those in experiment stations and elsewhere who are engaged in investigating the bacterial diseases of plants. I respectfully recommend that the paper be published as Bulletin No. 28 of this Division.

Respectfully,

Albert F. Woods,
Chief of Division.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

Page.

Introduction 7

Growth in fluid media 9

- Alkaline beef broth 9
- Acid beef broth 11
- Salted beef broth 12
- Acid vs. Alkaline beef broth 14
- Uschinsky's fluid 18
- Milk and litmus milk 18

Growth on solid media 20

- Loeffler's solidified blood serum 20
- Nutrient gelatins 21
- Nutrient agars 29
- Potato 33
- Coconut 35
- Radish 36
- White turnip 37
- Yellow turnip 38
- Rutabaga 40
- Carrot 41
- Sweet potato 43
- Sugar beet 44

Sensitiveness to acids 46

- Acid beef broths 46
- Lactic acid 48
- Potato broth 48
- Malic acid 49
- Cabbage juice 50
- Tomato juices 51
- Hyacinth broth 52

Feeble diastasic action 54

- Iodine starch reaction 55
- Growth on potato with addition of cane sugar 56
- Growth on potato with addition of maltose and dextrine 56
- Growth on potato with addition of diastase of malt 57
- Potato starch in peptone water with diastase 57
- Nutrient starch jelly No. 1 59
- Nutrient starch jelly No. 2 63
- Hyacinth starch jelly 64

Aerobism 65

- Fermentation tubes 65
- Growth in nitrogen 72
- Growth in vacuo 76
- Growth in hydrogen 79
- Growth in carbon dioxide 83

Page.
Relative nutrient value of carbon compounds 85
  Bouillon and peptone water with various sugars, etc 85
  Crude vegetable substances 86
  Sugar gelatin 87
  Sugar agars 87
  Sodium acetate 96
  Nutrient starch jelly with sugars, gums, and alcohols 96
Temperature experiments 98
  Thermal death point 98
  Maximum temperature for growth 102
  Optimum temperature for growth 108
  Minimum temperature for growth 108
Formation of acids 109
Formation of alkalies 110
  Rosolic acid test 110
  Acid fuchsin test 113
  Litmus 114
Reduction experiments 114
  Methylene blue 114
  Indigo carmine 115
  Litmus 117
Tests for hydrogen sulphide 127
Formation of indol 128
Tests for nitrites 129
  Peptonized beef broths 129
  Peptonized Uschinsky's solution 129
  Nitrate bouillon 129
Ferments 130
  Cytase 130
  Invertase 132
  Diastase 133
  Trypsin 134
  Lab ferment 134
  Oxidizing enzymes 134
Pigment studies 136
  The yellow color 136
  The brown pigment 140
Nature of the cell wall 143
Vitality 144
  Length of life in culture media 144
  Resistance to dry air 145
  Resistance to sunlight 147
  Resistance to heat 148
  Resistance to acids 148
  Resistance to alkali 148
  Growth in presence of calcium sulphite 148
  Growth over chloroform 148
Means of distinguishing the four species of Pseudomonas 149
Remarks on the yellow Pseudomonas group 152
  Characters in common 152
  Other species belonging to this group 153

ILLUSTRATION.

Page.

Fig. 1.—(a) Evolution of gas on adding hydrogen peroxide to potato culture of *Ps. phaseoli*.
(b) Uninoculated tube to which hydrogen peroxide has been added.. 135

THE CULTURAL CHARACTERS OF PSEUDOMONAS HYACINTHI, PS. CAMPESTRIS, PS. PHASEOLI, AND PS. STEWARTI, FOUR ONE-FLAGELLATE YELLOW BACTERIA PARASITIC ON PLANTS.

INTRODUCTION.

The morphology and pathogenic properties of *Pseudomonas hyacinthi* were described by Wakker in 1883–1889,[1] and were redescribed by the writer in 1897 in Proceedings of the American Association for the Advancement of Science,[2] and in 1901 in Bulletin No. 26 of this Division.[3]

The morphology, cultural characters, and the pathogenic properties of *Pseudomonas campestris* were first established by Pammel (in part), in 1895[4] and were more fully described by the writer in 1897[5] and 1898.[6] In 1898 they were described also by Russell and Harding,[7] and by Russell.[1] More recently Harding has shown that the disease produced by this organism occurs in cabbage in various places in Europe;[2] and Hecke has demonstrated its occurrence in Kohlrabi in southern Austria.[2a]

---

[1] (1) Vorläufige Mittheilungen über Hyacinthenkrankheiten. Botanisches Centralblatt, Bd. XIV, 1883, pp. 315–316. (2) Het geel-of nieuwziek der Hyacinthen veroorzaakt door *Bacterium Hyacinthi* Wakker. Onderzoek der Ziekten van Hyacinthen, en andere bol-en knolgewassen. Verslag over het jaar 1883. Haarlem, August, 1884. 8vo, pp. 4–13. (3) Onderzoek der Ziekten van Hyacinthen, en andere bol-en knolgewassen. Verslag over het jaar 1884. Haarlem, May, 1885. 8vo, pp. 1–11. (4) Onderzoek der Ziekten van Hyacinthen, en andere bol-en knolgewassen. Verslag over het jaar 1885. Haarlem, May, 1887. 8vo, pp. 1–5 and 27–37. (5) Contributions à la pathologie végétale: 1. La maladie du jaune ou maladie nouvelle des jacinthes, causée par le Bacterium Hyacinthi. Archives néerlandaises d. Sci. ex. et nat., Tome XXIII, 1889, pp. 1–25, pl. 1.

[2] Wakker's Hyacinth Bacterium. Proceedings of the Amer. Assoc. for the Adv. of Sci., 1897, p. 274.

[3] Wakker's Hyacinth Germ, *Pseudomonas hyacinthi* (Wakker). Washington, Government Printing Office, 1901, pp. 45, 1 pl., 6 text figs.

[4] Bacteriosis of Rutabaga (*Bacillus campestris n. sp.*). Bull. No. 27, Iowa Exp. Station, Ames, Iowa, 1895, pp. 130–135.

[5] (1) Science N. S., Vol. V, June, 1897, p. 963. Abstract of a paper read before the Biological Society of Washington, May, 1897. (2) *Pseudomonas campestris* (Pammel). The cause of a brown rot in cruciferous plants. Centralbl. f. Bakt. 2. Abt., Bd. III, July, August, and September, 1897, pp. 284, 408, 478, 1 pl.

[6] (1) The black rot of the cabbage. U. S. Dept. of Agr. Farmers' Bulletin No. 68, Jan. 8, 1898. (2) Additional notes on the bacterial brown rot of cabbages. Bot. Gaz., vol. 25, pp. 107–108. Amer. Nat. No. 32, p. 99. Both abstracts of a paper read before Soc. for Plant Morphology and Physiology in December, 1897.

[7] A bacterial rot of cabbage and allied plants. Wis. Exp. Station Bull. No. 65, Feb., 1898. (Issued in March.)

Pseudomonas phaseoli was described briefly and named by the writer in 1897,[3] after securing numerous infections with pure cultures. The disease which it produces had been previously ascribed to bacteria by Beach,[4] and by Halsted,[5] as the result of a microscopic examination, but the organism itself had not been described, nor had it been shown by means of pure culture inoculations to what organism the bean disease was due. Quite recently the same or a very similar organism has been described briefly by Delacroix, who obtained it from diseased beans in fields near Paris.[6]

Pseudomonas stewarti was found in sweet corn and described in 1897 by Stewart,[7] who, however, established its pathogenic nature only inferentially. It was named with some additional characterization by the writer in 1898 from a culture furnished by Mr. Stewart for that purpose.[8] Doubt still remains as to its pathogenic properties, and must continue until the disease has been produced with pure culture inoculations from this particular species and under conditions precluding its origination by any other organism. Of the existence of a disease of maize due to bacteria no one who has examined specimens from Long Island or elsewhere can have a moment's doubt. The question as to what species causes it can be settled definitely only by successful pure culture inoculations.

The following pages were originally intended to form part of Bulletin 26 of this Division, but the manuscript grew to such an extent under my hands, and came to include so many references to related organisms, that, finally, it was decided to add still more references of this character and to publish it separately, making this bulletin, as far as possible, a monographic or comparative study of the cultural characters of the yellow species of Pseudomonas parasitic on plants. This statement will serve to explain the arrangement of the text. Under each subhead *Ps. hyacinthi* is the organism first considered, but whenever comparative studies have made it possible statements are added respecting the behavior of related species. Occasionally mention is made of species not closely related, e. g. *Bacillus amylovorus*, *B. coli*, *B. carotovorus*, and at the end I have noted some other species which belong to this group of bacteria, and which I have here designated THE YELLOW PSEUDOMONAS GROUP.

---

[1] A bacterial disease of cabbage and allied plants. Proc. 11th, An. Conv. Amer. Col. and Exp. Stations, p. 86. (Issued in March, 1898.)

[2] Die schwarze Fäulnis des Kohls und verwandter Pflanzen, eine in Europa weit verbreitete bakterielle Pflanzenkrankheit. Centralbl. f. Bakt., 2 Abt., Bd. VI, 1900, No. 10, pp. 305–313.

[2a] Eine Bacteriosis des Kohlrabi. Zeits. f. das landw. Versuchswesen in Oesterreich, 1901, and subsequent letters to the writer. Inoculating from a pure culture furnished by Dr. Hecke, the writer has also recently produced the typical brown rot in cabbage.

[3] Description of *Bacillus phaseoli* n. sp. with some remarks on related species. Proc. Am. Assoc. for Adv. of Sci. for 1897, pp. 288–290.

[4] Blight of Lima Beans. N. Y. Ag. Exp. Station Bull. No. 48, new series, Dec., 1892, Geneva, N. Y., p. 331.

[5] A Bacterium of Phaseolus. Rept. of Bot. Dept. N. J. Exp. Station for 1892, pp. 283–285.

[6] (1) La graisse, maladie bactérienne des Haricots. Comptes Rendus, T. 129, p. 656. (2) Annales de l'Institut Agronomique, T. ——, p. ——.

[7] A bacterial disease of sweet corn. Bull. 130, Geneva Exp. Station, N. Y.; also 16th Ann. Rept. N. Y. Agr. Exp. Station for the year 1897, pp. 401–416.

[8] Notes on Stewart's sweet-corn germ, *Pseudomonas stewarti* n. sp. Proc. Am. Assoc. for Adv. of Sci. for 1898, pp. 422–426.

Some particulars have not been worked out as thoroughly as could be wished, e. g., (1) the relative nutrient value of nitrogen compounds, (2) the effect of antiseptics and germicides, but on the whole it seems best not to give any more time at present to these particular organisms, the main features of whose morphology and physiology have, it is believed, been made out correctly.

GROWTH IN FLUID MEDIA.

Alkaline Beef Broth.

In test tubes of Weber's resistant glass, containing 10 c. c. of 1:2 alkaline beef broth[1] the fluid always showed a feeble clouding in 48 hours when inoculated with a 2 mm. loop from a fresh fluid culture of *Ps. hyacinthi* and kept at 23° C., or thereabouts. Also, when the tubes were inoculated with a much smaller number of germs, viz, as few as could be transferred from a fluid culture on the extreme tip of a platinum needle, the clouding always followed, being a little delayed but in no way restrained. The importance of this fact will be apparent a little later when we come to discuss the effect of acid broths.

---

[1] This beef broth (stock 286b) was made as follows: Into a large beaker of Jena glass I put 1,100 grams of finely minced lean beef, covered it with 1,500 c. c. of distilled water (from a tin-lined copper tank), and set into the ice chest for 24 hours. The mixture was then strained as dry as possible through a clean towel which had been thoroughly washed in distilled water before using, an additional 800 c. c. of distilled water having been added previous to the straining. The result was 2,350 c. c. of red acid fluid. This was put into the steamer, warmed up to 100° C., and left at that temperature 45 minutes. It was then filtered through S. and S. paper, yielding when cold 2,000 c. c. of clear, pale, yellow fluid. This was then made up to 2,200 c. c. by adding distilled water. After thorough mixture of the broth and water by pouring, samples of the fluid were titrated against caustic soda, using phenolphthalein as indicator, 10 c. c. requiring 2.5 c. c. of $\frac{N}{10}$NaOH to exactly neutralize it. A fermentation tube filled at this time (25 c. c. of fluid) and afterwards inoculated with *Bacillus cloacæ* yielded 2 to 3 c. c. of gas, indicating the presence of muscle sugar. This acid fluid was designated 286a. To obtain stock 286b, 600 c. c. of this fluid was rendered exactly neutral to phenolphthalein by adding 7.5 c. c. of $\frac{2N}{1}$NaOH. On steaming one-half hour a slight precipitate came down. On filtering again the broth was perfectly clear and remained so. It gave a strong blue reaction with neutral litmus paper.

On the fourth day, in this alkaline beef broth, *Ps. hyacinthi* showed a small amount of yellow precipitate. On the 6th day there was less precipitate than in tubes of acid beef broth (stock 286a) 11 days old, but it was yellower. The clouding was so slight that a penholder was easily visible behind a thickness of two tubes.

On the eleventh or twelfth day there was more of the yellow precipitate than on the sixth, but it was not copious. Rolling clouds were visible on shaking, but no zooglœæ. There was no pellicle, but now for the first time a feeble rim of germs was to be seen on the wall of the tube at the surface of the fluid. Under a Zeiss hand lens (× 6 aplanat) this rim appeared as a pale amorphous membrane thickly set with a series of roundish colony-like aggregates, which were white or yellowish, and which did not dissolve when shaken down into the fluid. Four days later the largest of these colony-like bodies were distinctly yellow, the smaller ones being white. On the twentieth day the fluid was uniformly clouded; there was no pellicle, and no ragged zooglœæ were visible to the naked eye. The bright yellow precipitate on the bottom of the tube now covered a diameter of only 4 mm. The rim of germs was broad and filmy. It easily jarred off in large fragments, or as a whole, and fell to the bottom. It contained a great many zooglœæ set at regular intervals in what still looked under a × 6 Zeiss aplanat like a homogeneous membrane. The upper, larger, and older aggregates were decidedly yellow, and set so closely as to form a yellow border on the upper rim of the ring, which was exposed to the air. The lower, smaller, and younger zooglœæ on this ring were white, this part being submerged or barely out of the fluid. [Subsequent observations showed that these white zooglœæ always became yellow with increasing age and size.] The greater part of the clouding was still attributable to individual germs, but some small zooglœæ could now be seen in it, especially when examined with the hand lens. Under the compound microscope (Zeiss 16 mm. and 12 comp. oc.) the zooglœæ on the rim looked like small, closely set colonies on an agar plate, i. e., they consisted of roundish, colony-like bodies on a paler, homogeneous looking membrane. Stained with gentian violet and examined under high powers the homogeneous substratum was seen to be composed of slender rods, which were often in short chains of 6 to 12 or more segments, the individuals forming the chains being distinct and of the same size and shape as those not joined.

On the thirty-third day there was a moderately abundant yellow precipitate, and the color approximated Ridgway's canary yellow. The fluid was less cloudy than it had been, but was still uniformly so. It was not turbid with zooglœæ, but some small flecks were floating in it. There was no pellicle, but an easily detached, pale, fragile, homogeneous rim of germs, which was closely set with small, roundish, uniform-looking, colony-like aggregates. These did not dissolve readily in the fluid and all the larger ones were distinctly yellow and easily visible to the naked eye. The fluid had shown no acid reaction. It was now alkaline, and was not brown.[1]

On the fiftieth day the fluid was feebly and uniformly clouded, but much clearer than it had been. It was strongly alkaline to litmus; it was not ropy; there were no rolling clouds on shaking. There was no pellicle. The rim was 6 mm. wide and studded with zooglœæ; the largest of these were one-third mm. in diameter and yellow to the naked eye; the precipitate was still bright yellow and rather copious.

On the seventieth day the fluid was nearly clear, and there was no brown stain in it. It had evaporated from 10 c. c. to about 6.5 c. c. Eighteen days later the fluid was entirely clear.

On the one hundred and nineteenth day there was no brown stain, and large irregular crystals were present in the sediment.

ACID BEEF BROTH.

This broth was from the same stock as 286b, but no alkali was added. Its acidity was +25 of Fuller's scale, i. e., 25 c. c. of $\frac{N}{1}$NaOH would have been required to render 1,000 c. c. of this broth neutral to phenolphthalein. It was feebly acid to good neutral litmus paper. This fluid retarded growth slightly and was distinctly favorable to the formation of zooglœæ. The precipitate was more copious than in the alkaline beef broth and was duller yellow—a dirty Naples yellow. The clouding began in about 72 hours, when the inoculations were made with large loops from fresh fluid cultures and on the sixth day when the inoculations were made with as small a quantity of the fluid as could be lifted and seen on the tip of a platinum needle. Notes on one of eight cultures in this medium are given below:

Stock 286a, tube 11, February 4, 1898: Tube of resistant glass containing 10 c.c. of broth inoculated at 1 p. m. with *Ps. hyacinthi* from an alkaline beef broth culture (No. 1, January 29), which had been cloudy for three days and contained many actively motile germs. Only a tiny drop on the tip of a platinum needle was put into the tube, i. e., about 1/50 of a good-sized loop. February 5, clear; February 7, clear; February 8, clear. [Tubes exposed to the same temperatures as the alkaline beef broths.] Two check tubes of alkaline broth (286b) inoculated in the same way were cloudy on the third day. This broth exerts a distinct retarding influence which is especially noticeable when the dose of germs is small.

February 9. Clear.

February 10, 2.30 p. m. Very feebly clouded; some whitish flecks (zooglœæ) on the wall of the tube from top to bottom on one side.

February 19. Fluid turbid from numerous whitish flecks which are easily visible to the naked eye. (No zooglœæ visible in the two tubes of 286b held for comparison.) Rim well developed; most of its zooglœæ are white, but a few are yellowish. A pellicle consisting of zooglœæ held together by a film has gone to the bottom. Fluid homogeneous and now feebly alkaline to neutral litmus paper; in some of the tubes of this set the fluid has begun to clear a little at the top. Precipitate dirty yellow-white and rather abundant.

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[1] Throughout this bulletin "acid" and "alkaline" refer to litmus reactions unless it is otherwise stated.

March 12. No new pellicle; fluid uniformly thin cloudy; no crystals. The zooglœæ scattered through the fluid and lodged on the walls of the tube are very numerous, i. e., fifty times as many as in the two check tubes of alkaline beef broth. They consist of irregular, loose, rather large, whitish or very pale yellow-white flecks; rolling clouds are also visible on shaking. The tendency to form zooglœæ is much stronger in this fluid than in 286b, but they also form in the latter after a time. There is a thin rim of germs on the wall of the tube for a distance of 3 mm. above the fluid; this rim bears several hundred small, roundish, colony-like zooglœæ, most of which are now distinctly yellow—all the older larger ones. In the other seven tubes of this set most of this rim went down easily as a thin broken film on gentle shaking. The precipitate on the bottom of the tube covers a diameter of 10 mm. and is dull yellow—between wax yellow and Naples yellow. The color of the precipitate in the check tubes of stock 286b is brighter, and lies between gamboge yellow and chrome yellow.[1] The fluid is now plainly alkaline to neutral litmus.

April 13. Fluid nearly clear, no rolling clouds on shaking; no brown stain; moderately alkaline to neutral litmus paper. Precipitate more copious and certainly of a duller yellow than in the strongly alkaline broth. Rim of germs all of one kind, i. e., not contaminated, 6 mm. wide; all of the zooglœæ on it are roundish, but only the older and larger ones are distinctly yellow.

April 25. The fluid has cleared, and there is no brown stain in it; when boiled, a vapor was given off which immediately blued moist neutral litmus paper.

SALTED BEEF BROTH.

To determine the effect of sodium chloride upon *Ps. hyacinthi* the following experiment was instituted: Stock 529, which was an ordinary 1:2 acid beef broth (containing 1 per cent Witte's peptonum siccum and one-half of 1 per cent c. p. NaCl), was divided into two parts. To one was added an additional 1 per cent c. p. sodium chloride, forming stock 535; the other half was held as a check. The two culture fluids were then pipetted into clean test tubes of resistant glass and sterilized by steaming for a few minutes on each of three consecutive days. After some time the two sets of tubes, each of which contained exactly 10 c. c. of fluid, were inoculated at the same time and in the same way, i. e., with approximately equal numbers of bacteria from a well-clouded sugar bouillon culture (No. 6, October 29). Each of 6 tubes (3 of each sort) received a 2 mm. loop of the cloudy broth. The other 6 tubes each received as small a drop of the clouded fluid as could be seen distinctly on the end of a platinum needle. The experiment began at 3 p. m. November 5, 1899, and the subsequent observations were made at about the same time each afternoon. The following table, in which 0 denotes "clear," + "feebly clouded," and + +, "very feebly clouded," shows the date at which clouding took place in these tubes, the temperature being the same, i. e., 18° to 22° C.:

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[1] Ridgway's Nomenclature of colors, 1st ed.

Table I.—*Showing effect of sodium chloride on Pseudomonas hyacinthi in beef broth.*

Stock.	Method of inoculation.	Per cent of NaCl.	November—												No. of tube.
			6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	
529	Needle	0.5	0	0	0	# 0	0	+							1
529	Needle	0.5	0	0	0	# 0	++								2
529	Needle	0.5	0	0	0	# 0	0	+							3
535	Needle	1.5	0	0	0	# 0	0	0	0	0	0	0	++		4
535	Needle	1.5	0	0	0	# 0	0	0	0	0	0	0	0	0	5
535	Needle	1.5	0	0	0	# 0	0	0	0	0	0	0	0	++	6
529	2 mm. loop ...	0.5	0	# 0	0	+									7
529	2 mm. loop ...	0.5	0	# 0	0	+									8
529	2 mm. loop ...	0.5	0	# 0	0	+									9
535	2 mm. loop ...	1.5	0	# 0	0	0	0	0	0	++					10
535	2 mm. loop ...	1.5	0	# 0	0	0	0	0	0	++					11
535	2 mm. loop ...	1.5	0	# 0	0	0	0	0	0	++					12

There was a distinct slight retardation in stock 529, owing to the fact that no alkali was added to it other than that which was naturally in the peptone and perhaps, also, to the 0.5 NaCl which it contained. An examination of the table, however, shows that there was a very marked *additional* retardation in stock 535, which could be attributed only to the excess of sodium chloride. The retarding influence once overcome, growth proceeded, e. g., in tube 4 clouding was as twice as heavy on November 17 as on November 16, and in tubes 10, 11, and 12 it was twice as heavy on November 15 as on November 13. The date on which the clouding would have taken place in peptonized beef bouillon free from sodium chloride and neutralized to phenolphthalein by means of caustic soda is indicated in the table by # (see page 9).

ACID *vs.* ALKALINE BEEF BROTH.

The following experiments were undertaken in 1899 to determine, approximately, the limits of growth of *Ps. hyacinthi* in acid and alkaline media. As a standard for comparison, I made use of a peptonized 1:2 beef broth neutralized to phenolphthalein by caustic soda, and well adapted to the growth of this organism. Portions of this stock were then acidified with varying quantities of malic acid, and others were rendered alkaline to phenolphthalein by an excess of caustic soda. Each test tube was of resistant glass, contained exactly 10 c. c. of the fluid to be tested, and was exposed to the same temperature. Except −80, all were inoculated June 11, from tube 8, May 14, a coconut culture, the growth of which had been delayed for some weeks in a U tube (nitrogen). Each tube received a very large number of germs and approximately the same number, i. e., a scant 2 mm. loop of the fresh yellow slime. Except −80, all of the cultures were carried through in duplicate. The approximate date of the clouding (temperature 25° to 30° C.) is shown in the following table, in which 0, is "clear," +, "feebly clouded," and ++, "very feebly clouded."

TABLE II.—*Showing growth of Ps. hyacinthi in peptonized alkaline, neutral, and acid beef broth, inoculated June 11.*

Acid and alkaline marked in degrees of Fuller's scale.[a]	June—					July—	
	12	13	15	19	29	23	26
−80	0	0	0	0	0	0	0
−40	0 0	0 0	0 0	0 0	0 0	0 0	0 0
−20	0 0	+ + ?	+ +		Not one-fifth as much growth as in the following.		
0	0 0	+ + Several times as cloudy as the preceding.		Twice as cloudy as the preceding.			
+15 (Stock 473a, acid of beef broth).	+ + 0	+ +	Clouding closely like preceding.	If anything, cloudier than the preceding.	A little cloudier than the preceding.		Rather more growth than in the preceding.
+30 (This and the following were acidified with malic acid.)	0 0	0 0	0 0	0 0	+ 0	Abundant bright yellow rim in each. Fluid now alkaline to litmus. Organism is able to make a moderate growth when it has overcome the malic acid. More growth in rim and pellicle than 0 or +15. Less precipitate than in 0 broth.	
+60	0 0	0 0	0 0	0 0	0 0	0 0	0 0
+90	0 0	0 0	0 0	0 0	0 0	0 0	0 0
+120	0 0	0 0	0 0	0 0	0 0	0 0	0 0

[a] Each degree on this scale corresponds to 1 c. c. of $\frac{N}{1}$ acid or alkali per liter of culture media, i. e., +15 means that the fluid is acid, and that 1 liter would require 15 c. c. $\frac{N}{1}$ alkali to render it neutral to phenolphthalein; −20 means that the fluid is alkaline, and would require per liter 20 c. c. $\frac{N}{1}$ acid to neutralize it.

From this table it would appear that the limits of growth for *Ps. hyacinthi*, under the conditions mentioned, lie between —20 and —40 on the alkaline side (probably near —35) and somewhat beyond +30 (probably near +40) on the acid side. For a long time I was in doubt as to whether any growth had taken place in +30, and it is not at all improbable that with the introduction of a lesser number of germs—e. g., a loop from a fluid culture—no growth would have taken place.

At the same time duplicate tests were made of a number of other bacteria and some of the results obtained are shown in the following table. Here, again, the tests were insufficient in number to bring out all of the peculiarities of the organisms. For instance, there should have been broths with intermediate grades of alkalinity and acidity, and for two of the organisms, *B. pyocyaneus pericarditidis* and *B. coli*, the series should have been extended on the alkaline side to at least —100. I have partially compensated for this by stating how soon the clouding appeared in certain of the fluids. In case of *Ps. stewarti* and *B. coli* the experiments should have been repeated in the +60 broth, since the growth was in any event feeble, and I was at times in doubt as to whether there had been any whatever. The —80 bouillon was inoculated June 13 with 2 mm. loops from fluid cultures 2 days old.

TABLE III.—*Showing growth of various organisms in alkaline, neutral, and acid beef broth (peptonized) with date of clouding.*

Organism.	Reaction of bouillon to phenolphthalein.								
	−80	−40	−20	0	+15	+30	+60	+90	+120
Ps. hyacinthi			2nd day.	▨	▨	18th day or later.			
Ps. campestris		2nd day to 5th day or later.	▨	▨	▨	1st day or 2nd			
Ps. stewarti		1st day or beginning of 2nd	▨	▨	▨	1st day.	?		
B. pyocyaneus pericard	5th day or 6th day.	▨	▨	▨	▨	1st day.			
B. amylovorus			1st day.	▨	▨	1st day.			
B. coli	Between 6th and 16th day.	▨	▨	▨	▨	1st day.	?		

USCHINSKY'S FLUID.

This fluid proved to be a very poor medium for the cultivation of *Ps. hyacinthi*. If only a few bacteria were put in, the fluid remained clear. If more were put in, growth appeared, but clouding was retarded (sometimes as long as 18 days) and was never other than feeble. On standing several weeks, there formed a feeble rim, at first white, then yellow, and a translucent pellicle dotted with roundish yellow zoogloeæ, which became yellower. If the rim or pellicle was shaken down into the bottom of the tube while it was still pale, it never acquired any deeper yellow. The fluid was never more than feebly clouded. The precipitate was bright yellow, but very scanty, amounting at the end of a month to a breadth of only 2.5 mm. on the bottom of the tube. At the end of 2 months seven-tenths of the original fluid remained. It had cleared, was free from any brown stain, and contained no crystals.

Very delicate white films and woolly flocculent bodies formed in this fluid and never became yellow. Under the microscope these colorless shreds and films consisted of enormous numbers of short, slender, motionless rods, so united that when the cover glass was jarred the mass moved as a whole. At first these bodies were supposed to be contaminations. The rods, however, were of the right size and shape for *Ps. hyacinthi*, and when these films and flecks were removed to beef broth, potato, or other suitable media only this one yellow organism developed. These bodies seemed so remarkable that a year later the experiments in Uschinsky's solution were repeated, with, however, identical results. *Ps. campestris* and *Ps. phaseoli* also grew feebly in this solution and with retardation, but without the films characteristic of *Ps. hyacinthi*. On the contrary, *Ps. stewarti* grew in it for a long time, and very copiously. *Ps. hyacinthi* grew very much better in Uschinsky's solution when 1 per cent Witte's peptone was added to it. In 3 weeks the growth in this peptonized fluid was 100 times as abundant as in the check tubes.

MILK AND LITMUS MILK.

The milk was obtained from a clean dairy and its reaction was amphoteric. It was used, nearly free from cream, in 10 c. c. portions, in test tubes of resistant glass. It was sterilized (about 24 hours after milking) by subjecting it, in wire crates, to streaming steam for 15 minutes at 100° C. on each of 4 consecutive days, and none of the many check tubes ever spoiled.

Many tubes of milk were inoculated with *Ps. hyacinthi* at different times. All gave the same result. For some time there is no visible change other than the formation of a yellow bacterial rim or pellicle, or both, with some yellow precipitate. Accompanying this growth there is a slowly increasing alkalinity, which is first clearly visible on about the third to fifth day. Toward the close of the first week, or during the second week, a very slow separation of the casein (paracasein?) takes place. The first visible separation is usually apparent about the fifth to sixth or eighth to tenth day in the form of a millimeter-deep layer of clear whey on top of the milk, which is still entirely fluid. By the end of the third or fourth week the fine white casein has settled so that it occupies only about one-half of the fluid, the supernatant whey being pale yellow and transparent. Above this whey in old cultures there is always a 5 to 7 mm. wide, dense, bright-yellow bacterial rim on the tube. The casein does not set on the start and is never coarse flocculent. It finally becomes packed together in the bottom of the tube, but for a long time it consists of tiny separate particles which roll over each other easily when the tubes are shaken. This precipitated casein finally changes from white to yellowish and is slowly redissolved (peptonized). At no time during this precipitation and re-solution of the casein is there any acid reaction or any formation of gas. The whey from such cultures had a slightly bitter taste.

The reaction of the medium is best observed by adding to the milk enough litmus water to make it a deep lavender color; i. e., 10 c. c. of a saturated watery solution of c. p., blue, dry, lime-free litmus to 200 c. c. of milk. Many cultures were made in this medium with exact results. During the first 8 or 10 days the blue color very slowly deepens and the separation of the casein begins.[1] During the next 10 days or so the casein slowly settles and is still blue. Subsequently the litmus becomes more or less reduced, but at no time is there any appearance indicating the formation of any organic acid. When the litmus is not reduced the whey is pale wine red by transmitted light (normal color of the litmus), but is not red by reflected light. If after several weeks or months of growth such reduced or partly reduced cultures are killed by heating for 10 minutes at 56° C., and are then exposed to the air for some weeks, the color of the litmus returns. The undissolved casein is now distinctly blue and the whey is not red by reflected light.

Numerous tiny, white, centrally constricted, sheaf-like crystals of tyrosin appeared in old milk cultures. Crystalline plates presumed to be leucin also appeared.

Ps. campestris and *Ps. phaseoli* both act upon milk and litmus milk in much the same way. Neither produces any acid or gas. Both cause a slowly increasing alkalinity in the milk with the separation of the casein from the whey by means of a lab ferment. In both, a portion of this casein is redissolved (peptonized) with the formation of tyrosin and leucin. *Ps. stewarti*, on the contrary, does not precipitate the casein or produce any other visible change in the milk, not even after several months, although it forms a distinct bacterial rim and a rather abundant bright yellow precipitate. (For action of this organism on litmus in milk, see Reduction.)

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[1] In one instance whey appeared in two tubes of blue litmus milk the fourth day. See table under Reduction.

The kind of litmus used with milk sometimes has an important bearing on the results obtained, e. g., an acid reaction was obtained when *Ps. hyacinthi* was grown in milk colored with Sharp & Dohme's neutral solution of litmus, and, as this was the first litmus used, it might easily have led to erroneous conclusions had not further experiments been instituted with other brands of litmus. This litmus, which is very sensitive. keeps indefinitely, and is in use in many laboratories in this country, is preserved from deterioration (as I have since learned from the manufacturers) by the addition of 12 per cent ethyl alcohol. The acid which uniformly appeared in cultures of *Ps. hyacinthi* containing this litmus was not developed from the milk, but from traces of alcohol remaining in the medium after sterilization (see Formation of acids and Reduction—Litmus). This acid is volatile and smells like acetic acid. In some cases the acid which was produced from the alcohol inhibited the growth of the bacteria before the casein was precipitated, and this never did separate out (3 months).

A similar acid reaction was subsequently obtained by cultivating *Ps. hyacinthi* in blue litmus milk, to which drops of c. p. absolute alcohol had been added. In this case also only a slight amount of acid was formed, and it was not visible for some days. In both tubes the casein was thrown down by the lab ferment before there was any distinct acid reaction. Methyl alcohol was also tried in the same litmus milk, but no red reaction was obtained (56 days). The lavender-blue milk gradually became deep blue, and the whey separated slowly from the casein in the way already described. Evidently this organism can not break up wood alcohol.

For notes on the behavior in other fluid media, see Fermentation tubes, Sensitiveness to acids, Relative nutrient value of carbon compounds, Reduction of litmus, Formation of alkali, etc.

GROWTH ON SOLID MEDIA.

Loeffler's Solidified Blood Serum.

This medium was prepared from beef's blood in the pathological laboratory of Johns Hopkins Hospital under the supervision of Dr. Simon Flexner. It was solidified in test tubes in 15 or 20 c. c. portions, in long slants, with about 1 c. c. of fluid in the bottom of the V. The medium was in excellent condition for use, the surface being moist and the body of the substratum very light colored and clear. The slant surface of one of these tubes was streaked copiously, on June 5, from a coconut culture of *Ps. hyacinthi* 7 days old. Cultures of other yellow germs were laid on this medium at the same time, and all were kept in the dark at room temperatures. During the first 5 days the temperature ranged from 23° to 31° C.; during the next 20 days the range was from 22° to 34° (25° to 30° most of the time), and during the last 6 days, from 29° to 37° C.

Result.—On the fifth day *Ps. hyacinthi* showed an abundant, smooth, wet-shining, bright-yellow growth the whole length of the slant, and a copious yellow precipitate in the fluid. There was no liquefaction, or, if any, only the merest trace on one side at the bottom of the slant. In corresponding tubes of *Ps. campestris* and *Ps. phaseoli* there was a distinct liquefaction the whole length of the streak. On the seventh day there was a slight liquefaction under the streak. This was plainest in the lowermost part, but was not one-twentieth as much as in the corresponding tubes of *Ps. campestris* and *Ps. phaseoli*. On the fifteenth day the precipitate continued to be brighter yellow, and there was decidedly less liquefaction than in corresponding tubes of the two organisms just mentioned. The serum of the long slant preserved its normal shape and color very well, even in the air, only the middle part and the sides just above the fluid being sunken in and dissolved away. On a scale of 10, the ability of these three organisms to liquefy this serum was marked 7, 5, and 2, *Ps. phaseoli* liquefying it most readily and *Ps. hyacinthi*, least readily. On the thirty-second day the precipitate in the V was 15 mm. wide and 7 mm. deep, and was still a trifle yellower than in corresponding tubes of *Ps. campestris* and *Ps. phaseoli*, but the liquefaction was decidedly less. The serum under the fluid still preserved much of its original color and was not liquefied, free access of air being apparently necessary, in case of each of these three organisms, to the operation of the chemical changes ending in liquefaction. About one-half as much fluid was now present in this tube as in the corresponding tubes of *Ps. campestris* and *Ps. phaseoli*. This fluid was strongly alkaline.

On the same medium *Ps. stewarti* made an excellent bright buff-yellow growth, but there was no trace of liquefaction (32 days).

NUTRIENT GELATINS.

Ps. hyacinthi grew better on beef broth gelatins made strongly alkaline to litmus (neutral to phenolphthalein) than on those which received less caustic soda and were feebly acid or feebly alkaline to litmus. It grew well, however, in beef broth gelatin first rendered neutral to phenolphthalein and then feebly acidulated with malic acid. A still better growth was obtained by adding cane sugar to this acid gelatin, the best growth of all being with +48 and +54 malic acid gelatin with the addition of 5 or 10 per cent cane sugar. The composition of the three gelatins on which the best growth was obtained is given below:

(1) *Stock 205.*—1,500 gr. finely minced lean beef; 3,000 c. c. distilled water. Mixed and put into a cool box for 24 hours. Then in steamer 1½ hours at 70° to 90°

C., and finally at 100° C.; squeezed fluid through a clean towel, and steamed again for 1 hour. Filtered through S. & S. paper, flasked, and sterilized forming stock 204. Fermentation tubes of this broth (25 c. c.) yielded about 1½ c. c. of gas with *Bacillus cloacæ*, showing the presence of muscle sugar. 1,700 c. c. of 204+255 gr. gelatin (Coignet Père et Cie gélatine extra). Gelatin soaked in broth 1½ hours, then heated in steamer to 100° C., cooled, added whites of 6 eggs (previously neutralized with c. p. HCl) to clarify, steamed, filtered, flasked, and steamed, added 17 gr. Witte's peptonum siccum, steamed and filtered; 1,500 c. c. of acid fluid remained; when titrated with caustic soda, 3.4 c. c. $\frac{N}{10}$ NaOH were required to exactly neutralize 10 c. c., using phenolphthalein as indicator; i. e., the acidity was +34. Reduced the acidity to +25 by adding 500 c. c. of distilled water. Then added 25 c. c. $\frac{2N}{1}$ soda, and steamed, filtered, flasked, and sterilized. The gelatin was steamed as short a time as possible on each occasion. This stock contained about 13 per cent of gelatin.

(2) *Malic acid gelatin.*—680 gr. finely minced, lean beef; 1,500 c. c. distilled water. Mixed and put into the ice box for 24 hours. Boiled three-fourths hour, filtered, cooled to 70° C., added 3 gr. NaCl, 12 gr. Witte's pept. sic., 100 gr. of brown German gelatin, and when the gelatin was dissolved the whites of 6 eggs, which were incorporated by repeated pourings. Steamed 40 minutes at 100° C., filtered through flannel, and titrated with caustic soda and phenolphthalein—acidity +45. Measured out 300 c. c. into each of three flasks and exactly neutralized each to phenolphthalein by addition of 6.7 c. c. $\frac{2N}{1}$ NaOH. To one flask was then added 7.8 c. c., to another 10.4 c. c., and to the third 11.7 c. c. of a malic acid water, 1 c. c. of which exactly balanced 13.75 c. c. $\frac{N}{10}$ NaOH; i. e., enough acid so that the gelatins should be approximately +36, +48, and +54 of Fuller's scale. These three acid gelatins were then filtered, tubed, and sterilized by steaming for a few minutes on each of three consecutive days. These stocks contained 8 per cent of gelatin.

(3) *Stock 244c.*—2,000 c. c. distilled water, 1,000 gr. lean minced beef, 300 gr. gelatin (one-half white French, one-half brown German), 20 gr. Witte's peptonum siccum. The beef was soaked in 1,200 c. c. distilled water for 1 hour, then brought slowly up to 65° C. on a water bath with constant stirring, then steamed 1 hour and filtered. The gelatin was soaked for a few hours in 1,000 c. c. of distilled water and then added to the hot beef broth, along with the peptone. Steamed 45 minutes, filtered, added 50 c. c. water to make up the 2,000 c. c. Fluid acid to litmus. Titrated with caustic soda, using phenolphthalein as an indicator. 2.85 c. c. $\frac{N}{10}$ NaOH was required to exactly neutralize 5 c. c. of gelatin, i. e., the acidity was +57. 14 c. c. of $\frac{2N}{1}$ NaOH was then added to 500 c. c. of the medium to make stock 244c, which was then flasked and sterilized. This stock, which contained 15 per cent of gelatin, was not perfectly solid at 24° C. A fermentation tube of the beef broth (25 c. c.) used for this gelatin yielded several c. c. of gas upon inoculating with *Bacillus cloacæ* indicating the presence of muscle sugar.

To test the effect of varying grades of alkalinity, stock 244c was compared with three other portions of the same gelatin to which different amounts of caustic soda were added, viz, (1) with a gelatin containing, per liter, 40 c. c. less of $\frac{N}{1}$ NaOH (stock 244a); (2) with one containing, per liter, 20 c. c. less of the normal soda solution (244b); and, finally, (3) with one containing, per liter, 25 c. c. more of the normal soda solution (244d). These gelatins I commonly designate, following Mr. Fuller's scale[1] as +40 gelatin, +20 gelatin, 0 gelatin (neutral to phenolphthalein), and —25 gelatin. The litmus reaction of these gelatins was as follows: +40, feebly acid; +20, very feebly alkaline; 0, strongly alkaline; —25, very strongly alkaline.

As already stated, the best growth of *Ps. hyacinthi*, when peptonized beef broth was used, was in the 0 gelatin (stocks 205 and 244c), the next best was in the +20 gelatin. The difference in growth on these two gelatins was more striking at first than later on, the organism being able to partially overcome the inhibiting substances in the +20 gelatin. The growth in +40 and —25 gelatin fell far behind that in the other two. One was too acid and the other too alkaline.[2] These tubes were kept in a cool box at temperatures varying from 10° to 22° C. (most of the time 13° to 18° C.). In all the stab cultures the growth was best in the upper part, gradually fading out in the depths. The yellow color was also best developed near the surface, where there was freest contact with the air. There was no indication of yellow in the growth in the depths of the stabs.

The following notes represent the usual behavior of stab cultures in 15 per cent nutrient gelatin made neutral to phenolphthalein:

Stock 244c (10 c. c. of very clear gelatin in tubes 16 mm. in diameter): Stab made June 2, from a fluid culture 11 days old (cauliflower broth); one needle-thrust the whole length of the gelatin (5 or 6 cm.); tube kept in the cool box at temperatures varying from 10° to 22° C. (most of the time 13° to 18° C.). June 4 (range of temperature, 17° to 22° C.): A whitish thread distinctly visible one-half way down; it fades out gradually. June 5 (temperature, 13° to 15° C.): Stab whitish, feeble, visible three-fourths of the way down. June 8 (temperature, 13° to 16° C.): Stab begins to fade out two-thirds down; growth decidedly better than in the +20 gelatin—at least twice as much growth; slight liquefaction—i. e., a pit at the mouth of the stab, 3 mm. wide and 2 mm. deep. June 10 (temperature, 10° to 13° C.): Rather better growth than in the +20 gelatin, but fading out in the depths; no marked increase of liquefaction; surface growth pale yellow, rather dry looking, about 3 mm. in diameter; surface irregular. June 13 (temperature, 12° to 16° C.): A better growth than in the +20 gelatin, but fading out in the depths; surface growth about 4 mm. in diameter, pale yellow; pit of liquefaction 4 mm. wide and 2 mm. deep. June 18 (temperature, 14° to 18° C.): Growth a little better than in the +20 gelatin, but not now strikingly so; the stab fades out in the depths; surface growth pale yellow; pit of liquefaction only 5 or 6 mm. wide and 3 mm. deep; growth in the +40 gelatin is so slight as to be easily overlooked; growth in the —25 gelatin is confined to the surface and there is no liquefaction. June 28 (temperature, 13° to 19° C.): The pit of liquefaction is now 1 cm. deep and 1 cm. wide; it is a larger pit than in the +20 gelatin, and there is more growth in it, and also more in the depths of the stab, but the latter fades out at the bottom; the surface growth is distinctly yellow; in the +40 gelatin there is a barely visible growth in the upper part of the stab, a pit of liquefaction 3 to 4 mm. wide and 4 to 5 mm. deep, with a little whitish sediment at the bottom; in the —25 gelatin there is no visible stab, but a distinctly yellow surface growth 5 mm. in diameter, and a feeble liquefaction under it.

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[1] Fuller: On the proper reaction of nutrient media for bacterial cultivation. Jour. Am. Public Health Association, Oct., 1895, p. 381.

[2] For the varying behavior of *Ps. campestris* in these four gelatins, see plate in The American Naturalist, March, 1899.

The growth in the streak cultures was better than in the stabs. Even on the best gelatin and when large loops of fresh fluid cultures were used the streaks came up rather slowly, i. e., in 3 to 5 days, at 18° to 24° C. When 2 weeks old, these streaks were usually 2 to 3 mm. wide and 4 to 5 cm. long. The growth was pale yellow, not very dense, finely granular under Zeiss ×6 aplanat, and often fine crenulate-serrate along the margins. The streaks were made with a medium-sized oese (2 mm. diameter), and the germs did not show much tendency to spread beyond the original streak.

In streak cultures on stock 205 a much better growth was obtained than with concomitant cultures of *Ps. campestris*, *Ps. phaseoli*, or *Bacillus tracheiphilus*. In 14 days, at 22° to 24° C , the streaks were about 2 mm. by 5 cm. with fine crenulate-serrate margins; the surface was very pale yellow and finely granular under Zeiss aplanat. There was no liquefaction at this date, but 6 weeks later all of the gelatin (10 c. c.) was fluid except a little in the bottom of the tubes and in the extreme top of the slant, and there was a moderate amount of pale yellow precipitate. During this period the tubes were in a cool box at approximately 20° to 24° C.

Some interesting results were obtained with the malic acid gelatins. They did not inhibit growth, as fluids of the same grade of acidity would have done, and in the +36 gelatin growth was not retarded. In the +48 and +54 gelatin growth was slightly retarded at first, and in the depths of the latter it was never as vigorous as in the less acid media, the separate colonies in the lower parts of the stabs always remaining smaller. After a time, however, the growth in the upper part of the stab and on the surface of the +48 and +54 gelatin outstripped that in the +36. On the fifth day there was most growth in the +36 gelatin and least in the +54. On the eighth day the retarding influence was still visible in the +54 gelatin. Subsequently it was overcome except in the depths. Two other interesting differences were observed. In 56 days, at 17° to 20° C., there was no liquefaction whatever in the +36 gelatin, and the color of the organism was a very pale yellow. In the +48 and +54 gelatins, on the contrary, the surface growth was bright yellow, and liquefaction set in at the end of the third week, involving the upper one-third of the 10 c. c. of gelatin in the course of the next 10 or 12 days. At the end of 56 days two-thirds to three-fourths of the gelatin in these tubes had liquefied.

A repetition of the tests with the +36 gelatin gave no different results. In 40 days, at 8° to 20° C. (mostly under 15°), there was no liquefaction and no bright yellow color, although the stab was well developed and the surface growth was 7 by 8 mm. in breadth. Additional cultures in the +48 and +54 gelatin yielded no new or different results. After 40 days there was an abundant bright-yellow growth, and the upper one-third of the gelatin was liquefied to the walls. The remainder of the 10 c. c. of gelatin was unchanged, the stab fading out in the depths to scattered round white colonies.

It would seem, therefore, that excess of malic aid favored liquefaction and the production of the yellow pigment. Possibly, however, these results are to be ascribed solely to changes in the physical character of the gelatin. The melting point was slightly reduced by the addition of the acid and was lowest in the stocks which received the most acid.

The most growth obtained on any gelatin was with +54 malic acid gelatin to which 10 per cent of cane sugar had been added. This was a slant culture kept in the ice chest at 10° to 25° C., for 6½ months, during all of which time it was overlooked, so that if there was any retardation of growth at first, there is no record of it. When examined at the end of 6½ months, there was a most copious growth, but no trace of liquefaction. At some time during the summer the ice was allowed to get low and the gelatin melted, allowing a copious bright yellow surface growth to fall to the bottom. Subsequently, with the addition of more ice, the gelatin resolidified and a new surface growth formed. When examined at the end of the 6½ months, there was a dense bright yellow rim 12 to 15 mm. wide, and a copious surface growth separated from the yellow precipitate, already mentioned, by a mass of solid gelatin free from browning and clear, except for tiny scattered masses of bacteria imprisoned when the gelatin resolidified. The bright yellow surface slime was still alive.

In the +48 gelatin with 5 per cent cane sugar, at the end of 40 days, at 8° to 20° C. (mostly under 15° C.), there was a compact bright yellow surface growth, 12 mm. in breadth and a distinct stab, but no liquefaction. This experiment was repeated at 12° to 20° C., using both +48 and +54 gelatin with 5 per cent cane sugar and continuing the experiment 30 days. During this time there was no liquefaction whatever in the +54 gelatin and only a very feeble liquefaction in the +48 gelatin, i. e., the distinctly yellow surface growth, 5 or 6 mm. in diameter, was sunken in slightly. In another experiment with +48 and +54 gelatin with 10 per cent cane sugar, kept at 7° to 21° C. (most of the time above 14° and below 19°), there was no liquefaction in 49 days.

The same results were obtained with 0 gelatin (stock 244c), to which 10 per cent cane sugar was added. After 61 days at 15° to 20° C. there was no liquefaction whatever in one tube and only the very slightest in the other, and no brown stain in either. The growth was better than in tubes of sugar-free gelatin which liquefied.

There can be no doubt, therefore, that, while powerfully stimulating growth, cane sugar in small doses retards and in large doses entirely inhibits the liquefaction of the gelatin, whether the medium is acid or alkaline.

In all of the gelatins liquefaction took place very slowly. In the 0 gelatins it seldom appeared earlier than the sixth day and often not until after the fourteenth day (temperatures 13° to 18° C., 18° to 21° C., and also 22° to 24° C.), and much later on gelatins not so well adapted to its growth. Even when once initiated the peptonization of the gelatins proceeded at such a snail's pace that 6 to 8 weeks usually elapsed before the whole 10 c. c. became fluid. This very slow liquefaction could not possibly have been due to the amount of gelatin used in my cultures, since this varied from 8 to 15 per cent, or to the fact that in preparing the media the gelatin was boiled only a very short time so as not to injure its solidifying properties. Neither does the temperature at which most of the experiments were made (8° to 21° C.) appear to have been the retarding cause, since liquefaction was not more rapid at higher summer temperatures. To test this, a tube of stock 244c was inoculated in June, 1897, and left for some weeks at room temperatures. These ranged from 25° to 34° C., the temperatures nearly all of the time being above 27° C., and often for many hours 29° to 32° C., i. e., near the optimum for this organism. The following notes on such a culture may not be without interest:

Stock 244c.—Tube of 10 c. c. inoculated June 14, noon, with a large loop from a beef-broth culture made June 3. Temperature, 25° C., gelatin fluid.

June 15, 1 p. m. (temperature, 28° C.). Feebly clouded throughout, no clouding down from the surface on shaking. Not so cloudy as concomitant cultures of *Ps. phaseoli.*

June 16 (temperature, 29° C.): Much less cloudy than a corresponding tube of *Ps. phaseoli.* Hundreds of tiny zooglœæ have gathered into the top layers of the fluid gelatin and give to it, when shaken down, a granular appearance,

June 17 (temperature, 28° C.): Clear in comparison with a tube of *Ps. phaseoli.* Growth largely in shape of small zooglœæ, some of which are again gathering into the top layers of the fluid gelatin.

June 18 (temperature, 26° C.): Very slowly increasing cloudiness with some tendency of zooglœæ to gather into top layers of the fluid gelatin.

June 19, temperature 26° to 27° C.; June 20, temperature 29° C.

June 21 (temperature, 27° C.): A pale yellow rim on the tube at the surface of the gelatin. No pellicle, but some gathering of zooglœæ and individual rods into the upper layers. Not much precipitate. On gentle shaking the fluid clouds down from the surface.

June 28 (temperature since last record, 25° to 31° C.): A moderate amount of yellow precipitate, much more than on the 21st. A copious yellow rim on the tube at the surface of the gelatin. Gelatin nearly clear until gently shaken, when it clouds down from the aggregation of germs in the surface layers. Numerous small zooglœæ still visible. On putting into ice water the gelatin soon became solid.

July 8 (temperature since last record, 29° to 33° C.; i. e., very hot summer weather): Body of the gelatin nearly clear. A decided rim of yellow on the walls of the tube at the surface and some clouding of the upper layers of the gelatin. Considerable yellow precipitate. The fluid clouds down on shaking. It still solidifies quickly in ice water.

July 29 (temperature since last record, 26° to 34° C.): The gelatin has become clear. There is a yellow rim on the walls of the tube above the surface of the fluid and a copious yellow precipitate. The gelatin still solidifies in ice water, but it is only semi-solid at 15° C., and is perfectly fluid at 18° C.; i. e., the melting point has been reduced 6 to 8 degrees, indicating a partial peptonization.

The most rapid liquefaction obtained was with a streak culture on stock 205. It was inoculated with a large loop from a fluid culture 10 days old and was kept at 18° to 24° C. On the twelfth day there was a thin, pale-yellow streak 2 to 3 mm. wide and 5 cm. long, in the middle part of which there was a small hole containing fluid gelatin. This liquefaction began the ninth or tenth day with a slight sinking in of this part of the streak. On the twentieth day the liquefaction involved about one-fourth of the gelatin (10 c. c.). On the twenty-ninth day fully three-fourths of the gelatin was fluid and there was a copious pale-yellow precipitate (temperature since last record, about 22° C.). Not until the thirty-ninth day was all of the gelatin fluid.

All of these gelatins contained some muscle sugar, which may have slightly retarded liquefaction, since various writers have shown for a number of bacteria that small doses of grape sugar retard and large ones prevent liquefaction.

In stock 208 (stock 205 + enough $\frac{2N}{1}$ c. p. HCl to make it neutral to sensitive neutral litmus paper) there was no growth whatever at 10° C. This inoculation was a streak the whole length of a long slant. It was made with a large loop of fluid from a beef-broth culture 7 days old and well stocked with living germs, as shown by the result of concomitant inoculations into other media. This culture was kept under observation 22 days. Inasmuch as the organism grew well in stock 205, and will also grow at 10° C., the failure of this tube was ascribed to the sodium chloride developed in the gelatin by the addition of the HCl, enough being produced to give a feeble taste of salt. (See p. 13.)

In the + 20 gelatin (stock 244b) this organism and *Ps. phaseoli* behaved much alike, both growing very much better than *Ps. campestris.*

In poured plates (+ 20 gelatin in Petri dishes) the buried colonies were round, roundish, or ellipsoidal, with smooth margins. No spindle-shaped colonies were seen and none or few having rough, irregular margins. (See plate cultures under Nutrient Agars.)

The size of these buried colonies in densely crowded plates (2,000 to 3,000 colonies per field of Zeiss 16 mm. aprochromatic and 12 compensating ocular), after 5 days at 13° to 16° C., was usually 16 by 16μ or 16 by 20μ. Some, however, were smaller, and others were as large as 24 by 24μ or even 28 by 32μ. The colonies were nearly colorless and very finely granular, with margins sharply defined and free from irregular outgrowths. Occasionally there were queer looking compound colonies resulting apparently from the growth of the component members of small zoogl∞ae. The plates were distinctly clouded to the naked eye, but there was no liquefaction. Ten days later the colonies were decidedly larger, but otherwise much the same. The margins were still well defined and regular and there was no liquefaction. In less crowded plates of the same gelatin (200 to 600 colonies per field) at the end of 5 days (13° to 16° C.) the buried colonies were like those just described, only larger—28 by 28μ to 56 by 64μ, the greater number being 32 by 32μ to 36 by 36μ. Ten days later, at 12° to 16° C., the colonies had doubled in size, were round, roundish, or broadly elliptical, pale and finely granular (16 mm., 12 oc.), with clear, well-defined margins. The colonies in the deeper layers of the gelatin were decidedly smaller than those near the surface. The largest of the buried colonies, including some of the clumpy compound ones, were then a feeble tint of yellow. This color was only visible in the upper colonies. No spindle-shaped colonies were visible. Only two small pits of liquefaction were observed. These arose from surface colonies, of which very few were visible; i. e., the buried colonies did not easily break through and come to the surface, and free access of air appeared to be necessary for liquefaction.

None of the many pure cultures of *Ps. hyacinthi* in gelatin developed any gas bubbles (see Fermentation tubes), and the gas bubbles observed by Dr. Wakker in his gelatin cultures must be attributed to some contaminating organism.[1] Contrary, also, to Dr. Wakker's statements the gelatin did not become brown. In all of the gelatin cultures (tubes under observation from 3 to 6 weeks or more) it remained free from browning; i. e., was of the same color as when inoculated.

Ps. campestris and *Ps. phaseoli* both liquefy gelatin, and more readily than *Ps. hyacinthi*, but none of them are rapid liquefiers.

In nutrient gelatin stock 478, consisting of 1,000 c. c. stock 473b (beef broth acidity +17) and 100 grams of gelatin with 17 c. c. of $\frac{2N}{1}$ NaOH, *Ps. stewarti*, made a good growth. At the end of the forty-first day (temperature 17° to 22° C.) there was along the track of the needle puncture a thin line of growth, increasing toward the surface, and a dense, rather dry, and slightly roughened bright buff-yellow surface growth 7 mm. in diameter, but no liquefaction.

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[1] The only gas that ever appeared in any of my cultures was in one of four gelatin tubes made June 23, 1897, in Dr. Wakker's manner, i. e., directly from the yellow interior of a disorganized bundle in an otherwise sound bulb scale. This tube was inoculated with great care to avoid external contaminations, and it appeared to be all right for some time, but after 22 days a gas bubble appeared in the gelatin near the bottom of the stab (temperature 12° to 18° C.). This was then the only evidence of anything wrong, but two weeks later the nature of the contamination became perfectly plain, the gelatin becoming fluid to the walls of the tube in the upper two-thirds, the upper part of the liquefied portion being greenish fluorescent and the bottom covered with a copious whitish precipitate with a little of the yellow *Ps. hyacinthi* mixed in. Undoubtedly this was an aerial contamination, as *Ps. hyacinthi* is never greenish fluorescent.

The following may be noted as some of the most characteristic peculiarities of *Ps. hyacinthi* on gelatin culture media:

(1) Liquefaction in neutral, acid, or alkaline gelatins, made with peptone and beef broth containing muscle sugar, proceeds very slowly at all temperatures (8° to 32° C.), reaching out to the walls of the tube long before it has involved the whole of the gelatin in stab cultures.

(2) The addition of 5 or 10 per cent of cane sugar greatly favors the long-continued growth of the parasite and does not interfere with the development of the yellow pigment, but entirely prevents liquefaction, or reduces it to an insignificant phenomenon easily overlooked.

(3) An extremely superficial, whitish, chemical film appeared after some weeks around the surface growth, even when cane sugar was added (see Nutrient Agars).

(4) None of the gelatins showed any browning or other stain of the substratum.

(5) No gas bubbles appeared, except in one tube which turned out to be contaminated.

(6) Quite unlike strong growing facultative anaërobic species, such as *Bacillus coli* or *B. cloacæ*, the stabs always faded out gradually in the depths, being best developed near the surface, and least in the deeper parts of the gelatin.

(7) The separate colonies, which in many instances formed the lower part of the stab, were always round or roundish, never spindle-shaped, and were never distinctly yellow, i. e., they were white or whitish, the free access of air appearing to be requisite for the development of the bright yellow pigment.

(8) Even in Petri dish cultures the surface colonies developed better than the buried ones, and the buried colonies in the surface layers grew better than those in the deeper parts of the gelatin.

(9) Peptonized beef broth gelatin which is only neutral or feebly alkaline to litmus exerts a retarding influence on growth. The reaction for best growth of this species lies somewhere between +15 and 0 of Fuller's scale. Litmus neutral gelatin also exerts a retarding influence on several other plant parasites, e. g., *Pseudomonas campestris* and *Bacillus amylovorus*.

NUTRIENT AGARS.

(1) Streaks of *Ps. hyacinthi* on brown agar No. 207 (+22) yielded a good pale yellow growth and the same sort of crystals as cultures of *Ps. campestris*, viz, large compound X-shaped crystals of magnesium ammonium phosphate.[1] These crystals were, however, less abundant than in cultures of *Ps. campestris* of the same age, and this was attributed to a feebler production of ammonia. The streak was still pale yel-

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[1] The composition of this agar is given in *Centralblatt für Bakteriologie*, 2 Abt., Bd. III, p. 480.

low at the end of a month (living-room temperatures of March and April) and not at all sticky or gelatinous. Growth on this agar was retarded a little at first, but by the fifth day, when inoculated with large loops from beef-broth cultures a week old and kept at 21° to 23° C., there was a good, dense, yellow streak.

Fifteen months afterwards, a carefully preserved flask containing 500 c. c. of this same agar was opened and 100 c. c. of distilled water added to make up for what had slowly evaporated. The agar was then steamed, filtered, and filled into clean test tubes, forming stock 307. It was slightly browner and less elastic (more brittle) than when first made. Duplicate streak and stab cultures of this organism and also of *Ps. campestris* and *Ps. phaseoli*, both of which formerly grew well upon this agar, were made, using large loops of fluid cultures twelve days old. The loops were drawn lengthwise of the central part of the slant and were easily visible after the removal of the oese. *Ps. phaseoli* refused to grow on this agar, either in streaks or stabs (4 tubes, 56 days). *Ps. campestris* refused to grow in streak cultures and there was no visible growth in the stab cultures until after the sixth day (temperature 20° to 25°). This agar also exerted a powerful, restraining influence on *Ps. hyacinthi*. To the twelfth day the streak cultures showed no growth (temperature 20° to 25° C.). On the nineteenth day one of the streak cultures showed a distinct growth, but it was only 1 or 2 mm. by 10 mm. and was mostly in the agar. On the twenty-sixth day the streak measured only 25 mm. by 3 to 5 mm. The streak was dense, yellow, smooth, and wet-shining. The margins were thin and well defined. The organisms had grown down into the agar. On the fifty-sixth day the streak was 42 mm. by 5 to 8 mm. It was yellow, smooth, wet-shining, and contained several of the large X shaped crystals. No growth ever appeared in the other streak culture. Growth in the stab cultures was also much retarded and was very slow to appear upon the surface. This agar was not retitrated, to determine its acidity, but it was acid to neutral litmus paper, or, at least, not alkaline. When the moistened paper was dry, it seemed to be neutral.

(2) The following agar made by Mr. P. H. Dorsett was also tried:

1,000 c. c. of distilled water.
10 grams of Witte's peptonum siccum.
10 grams of agar.
2.5 grams of Liebig's extract of meat.

This fluid was cleared by the addition of the whites of 2 or 3 eggs and rendered moderately alkaline to litmus by the addition of carbonate of soda. It contained no muscle sugar and was +15.5 of Fuller's scale.

Repeated tests were made on this agar, usually in the form of streak cultures. There was no retardation of growth. The streak was distinct in 22 hours, at 27°, when the inoculation was made from a coconut culture 8 days old, and in 28 hours, at 22° to 28°, when the inoculation was made from an agar culture 13 days old. This growth was thin, distinctly yellow, smooth, wet-shining, translucent, homogeneous-looking, and not scanty. There were no down-growths into the agar, and the margins, while thin, were well defined, i. e., not nebulous. Even on recently slanted agar the organism showed little tendency to spread widely. The streaks remained translucent for a long time, a penholder being easily visible through them after a month or more. No crystals were formed and there was no browning of the agar even in old cultures. (An undescribed, white, endospore-bearing Schizomycete, isolated from rotting tomato fruits, browned this agar readily.)

After a month or two the streaks began to dry out, but the surface remained smooth, even in old cultures, and was homogeneous looking, except that, after some weeks, colonies of the same species frequently formed on the surface of the yellow slime. Tested on the seventeenth, forty-seventh, and fifty-third days, with neutral litmus paper, the slime was feebly to plainly alkaline. On the sixty-sixth day it was strongly alkaline. No acid reaction was ever observed.

An extremely thin, whitish, chemical deposit appeared on the surface of the agar beyond the streak, after a week or two, and slowly increased, being best developed on the lower part of the slant where the growth was best. This film dissolved in 10 per cent acetic-acid water in about one minute.

On the forty-seventh day the slime consisted of short slender rods, single or in pairs. Four rods joined end to end were rare, and chains were very rare. After a long search only one chain was found (about 10 segments). In none of these tubes did the growth increase much after the second week, and it never became what might be called copious. No reticulate or shagreen surface ever appeared in any of these cultures. (See Sugar agars under Relative nutrient value of carbon compounds.)

Streak cultures of *Ps. campestris*, *Ps. phaseoli*, and occasionally of *Ps. stewarti*, were made for comparison. The behavior of these three parasites on this agar was much the same as that of *Ps. hyacinthi*. All grew without retardation, and after a few days there was about the same amount of smooth, translucent yellow slime. No crystals were formed in the agar and no brown stain appeared, even in old cultures. The whitish chemical film appeared around the streaks whichever organism was used, and in some cases it was noted that it was best developed in the lower part of the streak. In case of *Ps. campestris*, this film was examined microscopically and found to consist of very minute granular bodies, which were readily soluble in 10 per cent acetic-acid water, but did not show any decided crystalline structure when examined with the polariscope.

In one series of tubes, after five days on this medium, *Ps. hyacinthi*, *Ps. campestris*, and *Ps. phaseoli* looked much alike, but the hyacinth germ was the brightest yellow and the cabbage germ the palest. On the seventeenth day *Ps. hyacinthi* was also noted as brighter yellow than the others. In another series of cultures the slime of *Ps. hyacinthi* was distinctly yellower on the sixteenth day than that of *Ps. campestris*, *Ps. phaseoli*, or *Ps. stewarti*. On the forty-seventh day the color of *Ps. hyacinthi* was saffron yellow (Ridgway, VI–4). The color of each of the other three organisms was paler, lying between buff yellow and chrome yellow. The cultures of these three organisms were also alkaline to litmus on various dates, and in cultures of the same age the slime on the seventeenth and forty-seventh day was more strongly alkaline than that of *Ps. hyacinthi*. All said, however, the cultures of all four of these organisms were much alike on this substratum at all stages of growth.

(3) Poured plates of *Ps. hyacinthi* were made in Petri dishes, using one of Mr. M. B. Waite's standard (litmus) neutral agars, in which *Bacillus amylovorus* had been found to make a good growth. In very crowded plates containing 8,000 to 10,000 colonies per field (Zeiss 16 mm. and 12 ocular), the agar, at room temperatures (25° C.), became milky cloudy on the fourth day. There were no distinct surface colonies, and the buried ones were irregular in outline, i. e., with ragged margins like the colonies of *Ps. campestris*. In a plate of the same age, but containing only about 600 buried colonies in each field (16 mm. 12 ocular), the colonies were larger, but otherwise of much the same character, i. e., roundish or somewhat irregular in shape with rough margins. No distinctly fusiform colonies were to be seen. Fusiform buried colonies were, however, observed in plate cultures made from Mr. Dorsett's agar.

In thin sowings of *Ps. phaseoli* on nutrient agar in Petri dishes (25 surface colonies and about 40 buried ones), on the seventh day (25° C.), the surface colonies were pale yellow, smooth, wet-shining, not piled up, and had thin, distinct margins. They were 1.5 to 4 mm. in diameter. The buried colonies were elliptical or bluntly pointed (0.6 to 0.7 by 0.3 to 0.4 mm.). The margin of the buried colonies was distinct but frequently a little roughened under the Zeiss aplanat. On the eleventh day some of the buried colonies were breaking through to the surface. The entirely buried ones were still small and elliptical, with either pointed or rounded ends. They were yellow in color and their margins were more or less roughened by small blunt projections. The surface colonies were now 3 to 8 mm. in diameter, smooth and wet-shining. Buried in the colony were a number of lighter and darker rings. The color was distinctly yellow, but pale rather than bright, i. e., somewhat like straw yellow. The margins were thin and well defined. Under high magnifications zooglœæ were visible in the colonies. There was nothing peculiar in the marginal growth and the individual rods on the margin were not very distinct (Zeiss 16 mm. and 8 mm. apochromatics with compensating oculars up to No. 18).

POTATO.

More than 100 cultures of *Ps. hyacinthi* have been made on potato. This medium was usually prepared by steaming slant cylinders (5 to 6 cm. long by 1 to 1.3 cm. thick) in well plugged clean test tubes of resistant glass, in 1 to 3 c. c. of distilled water. Occasionally I made use of drier cylinders, only the curved bottom of the test tube being filled with water. The potatoes found in the Washington markets usually bear three steamings of 15 or 20 minutes each without cracking open or losing their smooth surface and white color, and, if they are prepared beforehand in a cleanly way, this short cooking on 3 consecutive days is sufficient to render them sterile.

The color of the organism on this substratum varies from bright yellow to pale or dirty yellow. Usually the color is distinctly brighter than in corresponding cultures of *Ps. campestris* or *Ps. phaseoli*. During the first week or two in most cases the color may be said to approximate Ridgway's Indian yellow (VI–5); i. e., it is nearly as bright as gamboge. As the culture becomes old the color dulls. In well-grown cultures not too old the color approximated Ridgway's wax yellow (VI–7). The color of the slime from a typical potato culture 30 days old was exactly Ridgway's gallstone yellow. Frequently the germs from very old cultures were brownish yellow in mass. The slime from a culture 48 days old was between ocher yellow and tawny olive.

Usually, at temperatures of 20° to 25° C., in inoculations made from broth cultures, the bacterial mass was not plainly visible along the streak until after 2 or 3 days. In one case it was distinctly visible in 24 hours, but then the temperature was 28° C., and the inoculation was with a mass of yellow slime from the surface of a potato culture. After a week or two the germ appeared in potato cultures as a thin, rather feeble, wet-shining, pale yellow or bright yellow growth, covering a part only or nearly the whole of the exposed potato, but showing no inclination to fill up the water.

There is, of course, a moderate clouding of the fluid around the cylinder, and after some days or weeks there is a scanty yellow precipitate which does not increase (14, 24, 41 days). All distinctly yellow growth is restricted to that part of the cylinder above the water. This growth is so thin that very often the slight irregularities of the surface of the substratum are not obscured and, as the fluid evaporates, the bacterial layer shows no tendency to follow down the sides of the cylinder and occupy the exposed surface of the potato. There is never any filling up of the fluid with yellow slime, such as always appears in potato cultures of *Ps. campestris* and *Ps. phaseoli*. In comparison with either of these species the growth of *Ps. hyacinthi* on potato lags far behind, e. g., at the end of 2 days at 20° to 25° C. it is not one-twentieth as much, and at the end of 2 weeks it is not one one-hundredth as much. After the second week the hyacinth germ shows very little increase of growth on potato, whereas the other two germs continue to multiply for many days, converting the fluid in the bottom of the tubes into a solid mass of yellow slime even when as much as 2 or 3 c. c. of water is present (see Tafel VI, fig. 4, Centralb. f. Bakt., 2 Abt., Bd. III). This feeble growth on potato serves as a ready means of distinguishing this organism from the cabbage germ and the bean parasite, but not from some other yellow bacteria, e. g., *Ps. stewarti*.

The surface of this yellow growth, in *Ps. hyacinthi* even after several weeks, is usually homogeneous, smooth, and wet-shining. Very rarely, after the third week, I have seen a shagreen surface on the extreme upper part of the potato cylinder. After a few weeks (3 to 4) the bacterial layer is slightly sticky, often stringing up 1 to 2 centimeters when touched with the loop. After 3 or 4 weeks, when a considerable portion of the bacterial layer may be presumed to be dead, pale yellow, smooth, shining colonies, 1 to 3 mm. in diameter and gradually rounded up from the margin to a rather thick center, are sometimes seen dotting the surface. Zooglœæ occur in this slime, at least after some weeks (30, 37 days), even when they are not visible in the form of shagreen. In one culture which was examined microscopically in water on the thirtieth day they consisted of numerous tiny ragged aggregates of 10 to 100 or more individual rods.

All the cooked potatoes I have ever tested have been feebly acid to litmus. This acidity is overcome by the growth of *Ps. hyacinthi*, the fluid first changing to feebly alkaline, and then becoming and remaining strongly alkaline (13, 22, 24, 30, 37, 56, 67 days).

The substratum out of the water is changed (as happens in case of many other bacteria) to a pale gray within a few days, and this color extends downward slowly into that part under the water, until after 3 or 4 weeks all is grayed; usually by the eighth or ninth day the gray color extended down under the water 1 centimeter. This color is a pale smoke gray, lighter than Ridgway's smoke gray (I–12). Its depth of color varies in different cultures, depending apparently on slight chemical differences in the potatoes used. The fluid in the bottom of these tubes remained free from color for a time, but after 3 or 4 weeks it became feebly browned. This brown color was distinct enough to be detected without check tubes, but it was never more than a weak stain (67 days).

The cylinders were firm and resistant between the fingers, even after the hyacinth organism had grown on them for 6 or 8 weeks, and their cellulose was certainly not acted upon to any marked degree. The starch was also but little affected (see Feeble diastasic action). In young cultures there was no smell; in old cultures there was a feeble odor.

Ps. stewarti behaves on potato much like *Ps. hyacinthi.*

Potato cylinders on which *Ps. campestris* and *Ps. phaseoli* have grown are somewhat softened as if the middle lamella of the cell wall were attacked.

COCONUT.

This substratum was made by putting clean, washed slices of coconut flesh into sterile, cotton-plugged test tubes, adding 1 or 2 c. c. of distilled water (from a tin-lined copper tank), so as to cover the lower one-third or one-half of the slice, and steaming 15 or 20 minutes on 3 consecutive days. The coconut flesh contains no starch and very little grape sugar (reducing substance), but is rich in oil. With the exception of rice it is the whitest culture medium known to the writer. All the yellow germs which I have tried make a satisfactory growth on this medium, and owing to its whiteness the contrast in color is very striking.

Ps. hyacinthi grows on this substratum without retardation. Cultures at room temperatures of 20° to 25° C. usually appeared in 36 to 48 hours, when not too sparingly inoculated, and made a good growth in 3 or 4 days. Growth continues for several weeks and usually becomes quite abundant (in one culture on the fiftieth day the bright yellow slime was over 1 mm. deep), but the organism shows no tendency to thicken the fluid or make it yellow, or to cover the submerged parts, any more than on potato, and there is little precipitate. The growth on this medium is smooth, wet-shining, and homogeneous. It is not sticky except in old cultures, which sometimes string up slightly. After 50 days in the ice chest the bacterial layer was not noticeably sticky, but it dissolved slowly in water and then lifted up 1 cm. when touched with the loop.

The color of *Ps. hyacinthi* on coconut is bright yellow. After 7 days' growth on coconut the organism was yellower than an equally good growth of the same age on turnip. At the end of the same period it was decidedly yellower than a corresponding culture of *Ps. campestris.* After 7 days and 25 days its color was about the same as that of a corresponding tube of *Ps. phaseoli.* After 49 days the color differed, if at all, from the color of a corresponding tube of *Ps. phaseoli* in being a trifle brighter, i. e., in containing less orange.

After 50 days at room temperatures of 18° to 27° C. its color was between Ridgway's lemon yellow and his gamboge yellow (VI–10 and 11). After the same period in the ice chest, at 7° to 15° C., its color was between canary yellow (light cadmium) and chrome yellow (Ridgway, VI–8).

No spores could be found in a culture which had grown in the ice chest for 50 days.

A culture at room temperatures was feebly alkaline to neutral litmus after 50 days. A culture which had been kept in the ice chest for the same length of time, and consequently was not so far advanced in growth, was distinctly alkaline, i. e., more so than the preceding. After 81 days in the ice chest a culture was strongly alkaline to neutral litmus.

No acid reaction was observed.

No brown pigment was developed. After 49 days at room temperatures the substratum was as white as when inoculated.

No cytohydrolytic action was observed. After the organism had grown on it for 81 days (ice chest) the substratum was as tough as when first inoculated.

No crystals were observed and there was no decided smell.

Ps. stewarti grew in much the same way on this substratum, but frequently made less growth. The color of its slime was buff yellow, and crystals were formed.

RADISH.

Slices of small, tender, red-skinned, turnip-rooted radishes were prepared in the same way as the potato.

On this substratum the hyacinth organism made a good growth, as the following record shows:

Stock 211.—Much water. Inoculated February 19, 5 p. m., from a beef-broth culture 14 days old, and kept at room temperatures.

February 22, 3 p. m. A good growth on the surface above the water, pale yellow, wet-shining. Fluid clouded, no precipitate.

February 26. A wet-shining, pale yellow growth over the whole exposed surface. A good growth, but not more copious than that in a corresponding tube of *Ps. campestris*. A moderate amount of precipitate. This is a yellower germ than *Ps. campestris*. It shows so, plainly, on all four media (radish, turnip, carrot, and coconut).

March 5. A copious growth. No brown pigment.

March 16. No brown stain.

April 9. The culture has begun to dry out, but there is still about one-half c. c. of fluid in the bottom of the tube. There is a thin pale-yellow precipitate. The substratum has changed color decidedly. The check tubes are still white, but the substratum in this one is of a color not easily described, i. e., unlike any in Ridgway's color system. It approaches his raw Sienna (V–2), and if that color had in it a very slight amount of brown it would closely resemble the color of this substratum. On long standing, therefore, a brownish pigment appears in tubes of radish.

A year later this experiment was repeated, using globose red and oblong white radishes. The results were substantially the same. There was a copious, very wet-shining, very pale-yellow growth, which never became bright yellow like that on coconut. In each case the substratum finally became brown, but this change took place very slowly, and the color never became deeper than a pale russet (64 days).

The slime was feebly alkaline on the thirty-fourth and sixty-fourth days whichever medium was used.

WHITE TURNIP.

Slices from the roots of smooth, green-leaved (nonglaucous), flat-bottomed, edible, white turnips were prepared in the same way as the potato cylinders.

The hyacinth organism grew well on this substratum and without any marked retardation. On the third day, at 21° to 23° C., the growth was very feeble in comparison with that of *Ps. campestris* or *Ps. phaseoli.* On the seventh day, at 20° to 23°, the growth was copious over the whole of the exposed part of the cylinder and the fluid was very cloudy, but there was little or no precipitate. On the twenty-second day growth was copious in the air and also in the upper part of the water, i. e., there was a better growth than in corresponding tubes of potato. After 54 days there was still a copious growth.

The surface of the slime was smooth and wet-shining, even in old cultures (54 days).

After 7 days at room temperatures the color in one tube was pale yellow, except the scanty precipitate, which was canary yellow. After 22 days the same culture was pale yellow. In another tube, on the seventh day, the color was unlike any in Ridgway's book, but approximated his Naples yellow (VI–18). This slime was plainly yellower than the equally copious growth in a corresponding tube of *Ps. campestris.* At the end of 25 days the slime in the upper part of the tube against the glass had developed a pale reddish-yellow color, quite in contrast with the color of a corresponding tube of *Ps. phaseoli.* There was also the merest trace of this color in the first cultures on radish. After 50 days at room temperatures the slime in one tube was "dirty yellow," while in another it was "pale yellow," i. e., much paler than in a culture of the same age on coconut. In mass, on white paper, this pale yellow slime was between Ridgway's buff yellow and maize yellow (VI–19 and 21).

After 54 days, at room temperatures, the slime showed no alkaline reaction, but was plainly acid to neutral litmus paper (only one tube tested). This red reaction was apparent at once and became stronger as the paper dried.

A brown stain slowly developed in the substratum, being clearly visible only after 2 or 3 weeks. On the twenty-second day and the thirty-eighth day the substratum was not browned as much as in corresponding cultures on yellow turnips. On the thirty-eighth day the color in one tube approximated Ridgway's russet. In another culture of the same age the brown was paler, approximating his tawny olive. On the forty-ninth day the substratum was darker than on the nineteenth, and was several shades darker than in a corresponding tube of radish. At this time the color was approximately burnt umber (R. III–8), but it was a trifle lighter than that color and appeared to have a trace of red in it.

The stain in old cultures was always a distinct but feeble brown and differed from the stain of *Ps. campestris* principally in being a shade or two lighter.

YELLOW TURNIP.

This medium was made in the same way as the preceding. The turnips were of the same habit of growth as the white ones but were sweeter to the taste and were distinctly yellow. The relative amount of sugar in the two kinds was not determined.

Ps. hyacinthi grew remarkably well on this substratum and without any marked retardation. At room temperatures of 18° to 25° C. the bacterial layer was usually visible on the third day. A week after inoculation growth in the air was "copious" to "very copious," and growth in the water had been sufficient to produce a sirupy liquid. This growth continued for several weeks, entirely hiding the aerial part of the cylinder and converting all of the fluid (1 to 2 c. c.) into a solid slime which would not flow. In one tube, at the end of 8 days, there was 100 times as much growth as in corresponding cultures on potato. In other words, the organism behaved on this substratum exactly after the manner of *Ps. campestris* and *Ps. phaseoli* on potato.

At room temperatures the growth was smooth, wet-shining, and homogeneous-looking from the start, and it remained so for 2 months. There was never any shagreen surface or other surface indication of zoogloeæ; nor was the dense copious slime sticky (eighth day).

The color of the slime was pale yellow; i. e., distinctly paler than on some other media. Examinations of 4 different cultures on the third, fifth, seventh, eighth, twelfth, twentieth, twenty-second, and thirty-second days all agree in this particular. On the fifth day the slime was a little brighter than Naples yellow. On the eighth day the color of the slime from another tube closely resembled Naples yellow, but was lighter yellow than the slime from a corresponding culture on carrot. In one instance the precipitate was canary yellow, while the aerial slime was paler yellow. On the fifty-fourth day, viewed without removal from the tube, the slime appeared to be russet color, but on putting a mass of it on white paper and comparing with Ridgway's plates its color was ochraceous.

On the eighth day the slime was distinctly alkaline to neutral litmus paper (one tube only was tested). In another tube, on the fifty-sixth day, the slime was feebly alkaline.

No stain of the fluid or of the substratum was visible during the first week of growth, but during the second or third week a brown color appeared and slowly increased in depth. On the twenty-second day this color resembled tawny olive, but was paler. On the thirty-second day it was still only a pale brown. After 69 days the color of this pigment was between Ridgway's russet and burnt umber.

Bacillus amylovorus made only a moderate growth on this substratum, and produced no brown stain, but developed an acid.

After a year or two this test of *Ps. hyacinthi* was repeated at 20° to 25° C., using yellow globe turnips (a rough-leaved, nonglaucous sort). On the third day 5 sq. cm. of the slant surface was covered with a smooth, wet-shining slime, which was abundant enough to hide the substratum. On the seventh day there was a copious yellow, smooth, wet-shining growth over the whole cylinder and in the water, but no browning of the substratum. On the eighteenth day the fluid was so full of the yellow slime that it would not flow when turned bottom up, and there was a slight browning of the upper part of the substratum. On the twenty-seventh day there was a distinct pale-brown stain in the upper part of the substratum. On the thirty-fourth day the slime was neutral to neutral litmus paper. On the fiftieth day the color was between burnt umber and mummy brown, and the fluid was grown solid with the yellow-brown bacteria. On the sixty-fourth day the color of the substratum was burnt umber. The culture had a faint, peculiar smell. The outline of the substratum was preserved, but on being removed from the tube it was mushy soft to the fingers, and even to a piece of litmus paper which could be thrust into it. The substance was feebly alkaline throughout. There were some involution forms, but nothing resembling spores. Large crystals were present.

No starch remained, if any was originally present. The middle lamella was dissolved or greatly softened. The cell wall proper (of the turnip) was apparently intact, but for the most part the contents of the cells were gone, although some large and small rings of doubtful origin remained. With Russow's cellulose test many of these cells of the substratum did not stain at all, a few became deep blue, and a few deep purple. In most, the walls remained colorless, but the contents of the cells reacted pale blue. Corresponding results were obtained with chlor-iodide of zinc. The contents of the cells frequently became blue while the walls remained colorless or turned to brown or reddish brown. Doubt was thrown on these results, however, by the behavior of the check tubes, which also gave an uncertain cellulose reaction with these reagents; i. e., cell walls purplish in the chlor-iodide of zinc (on long soaking), and bright blue only in a few cells and parts of cells with Russow's test.

Ps. campestris also made a prompt and copious growth on this substratum, but there were some differences. On the seventh day the growth, while very abundant, was scarcely distinguishable in color from the substratum; i. e., it was plainly less yellow than that of *Ps. hyacinthi*. At this date the fluid was grown full of the bacteria (solidified). On the eighteenth day the whole substratum was browned and this color was a much deeper brown than in the corresponding tube of *Ps. hyacinthi.* On the fiftieth day the color was burnt umber, and on the sixty-fourth day dark burnt umber. The slime was neutral or slightly alkaline on the thirty-fourth day, and feebly alkaline on the sixty-fourth day. The tissues were softened.

On this substratum *Ps. stewarti* made a thin buff yellow, slightly iridescent growth. On the seventh day there was one-fifth as much growth as in corresponding tubes of *Ps. hyacinthi* and one-tenth as much as in *Ps. campestris.* Growth did not increase much after the first or second week, and there was no browning or softening of the substratum. The culture was alkaline on the thirty-fourth and sixty-fourth days. After a time the water surrounding the turnip contained a moderate amount of buff yellow precipitate, but it never became thick or solid from excessive multiplication of the bacteria.

RUTABAGA.

Test-tube cultures of this turnip (which had smooth glaucous leaves) were prepared with distilled water in the ordinary way (see Potato). The tests were made at the same time and in the same manner as on the yellow globe turnip, and the results were much the same.

With *Ps. hyacinthi* the growth was copious from the start, and not only covered the cylinder, but filled the fluid (solid). There was no stain of the substratum until after the twenty-seventh day, but this was covered by the bacterial growth so as not to be exposed anywhere directly to the air. On the fiftieth day the bacterial slime exhibited a smooth, wet, dirty, brownish yellow surface. The upper part of the substratum was now browned. The slime was acid to neutral litmus, leaving a distinct reddish color as it dried, and the cylinder was softened so that it mashed easily with a glass rod. The fluid was still plainly acid after adding 25 c. c. of water and stirring. On boiling only a trace of acid was given off in the steam. On continuing the boiling so that the fluid was reduced to 6 c. c. it was more strongly acid, and the acidity became still more pronounced on reducing it to 3 c. c. The boiled fluid had a slightly bitter taste. There was a slow evolution of gas and no white precipitate when this rather thick slime was put into barium chloride water (acid).

On this substratum *Ps. campestris* grew very promptly. By the seventh day the fluid was grown solid and the cylinder in the air bore on all parts a very copious, wet, shining, smooth, yellow growth. At this time there was already a slight stain of the substratum. This stain became more pronounced and extended to the whole substratum on or before the eighteenth day. This color (slime and substratum) gradually deepened through raw umber (fiftieth day) to mummy brown (sixty-fourth day). On the thirty-fourth and sixty-fourth days the thick slime was acid to litmus, especially when diluted with distilled water. The tissues were softened and there was a peculiar smell, which was not rank or strong.

The behavior of *Ps. stewarti* on this substratum differed from that of *Ps. hyacinthi* and *Ps. campestris* in the same way as on the yellow globe turnip and was even more pronounced, so that it might be used as a means of distinguishing these organisms. The growth on the seventh day was about one-tenth as much as *Ps. hyacinthi* and one-twentieth or one-thirtieth as much as *Ps. campestris.*

On the eighteenth day the differences were as follows:

Ps. stewarti: Growth, buff yellow, thin, covering the whole of the air-exposed surface, but not dense enough to hide the slight irregularities of the substratum (not smooth). Surface slightly iridescent and with fine striæ (Zeiss × 6 aplanat), precipitate buff yellow and moderate in amount, water not grown full of the solid yellow slime, substratum not browned.

Ps. hyacinthi and *Ps. campestris:* Slime in the air copious, smooth, very wet-shining, pale yellow, surface not iridescent. Fluid grown full of the yellow slime (solidified). Substratum browned or ready to brown.

In old cultures of *Ps. stewarti* there was no increased growth, no brown stain, and no softening of the tissues. On the thirty-fourth day the thick slime would not wet litmus paper until water was added, when it gave an alkaline reaction. On the sixty-fourth day there was "a peculiar smell" and a feebly alkaline reaction. The iridescence persisted.

CARROT.

Cylinders of carrot were prepared in the same way as the potato cylinders.

Ps. hyacinthi grew well on this medium at 20° to 23° C., and generally without any distinct retardation. Usually growth was visible on the third day, and continued for several weeks, covering the aerial part of the cylinder with a bacterial layer a millimeter thick. The fluid in the bottom of the tube (1 to 2 c. c.) was also filled with a thick yellow slime, so that after 3 weeks it could usually be turned bottom up without flowing. Generally, though not always, growth was copious enough by the end of the first week to obscure the orange red of the substratum, which was not the case with *Ps. campestris.*

The surface was always wet-shining, even in very old cultures. In some it was smooth and homogeneous-looking from the start, and remained so. In others the surface was shagreened at first, but after eight days became smooth and homogeneous-looking. The bacterial slime was not sticky on the eighth day, in which particular it is very unlike *Bacillus tracheiphilus.* Subsequently (thirty-first and sixty-seventh days) it became slightly sticky.

The color on the fifth day was "bright yellow." On the eighth day it was between chrome yellow and maize yellow (R. VI–8 and 21). These two colors are compounded of varying amounts of orange cadmium, pale cadmium, and white. The slime in old cultures became darker, as if from admixture with a brown stain. On the thirty-first day the color was between ochraceous and raw sienna (R. V–2), being near the latter color. After sixty-nine days the slime in one tube was noted as "dark yellow" and in another as ochraceous to tawny ochraceous. In one of these tubes the carrot was observed to be decidedly deeper orange than when it was inoculated; i. e., than check tubes. On the fourteenth day the color and general appearance of this slime closely resembled that of a culture of *Ps. phaseoli* made for comparison.

On the eighth and twenty-third days the slime was distinctly alkaline to neutral litmus paper. On the same day the slime was more alkaline in a tube 31 days old than in one of the same age and origin, but in which the organism had grown for only 23 days; i. e., was restrained from growth by heat during the first week. In a culture 67 days old the slime was plainly alkaline.

After two and one-half months' growth, the carrot cylinders retained their form perfectly (2 tubes), but went into pulp easily under pressure of the fingers, as if the middle lamella had been partially dissolved. These cylinders had a soapy-feeling, and a feeble but distinct smell suggestive of ammonia and amin compounds.

In one instance crystals or crystal-like bodies were observed in the slime of old cultures (69 days).

Penicillium grew readily on carrot covered by this organism, was found associated with it in a number of the bulbs received from the Netherlands, and is mentioned by Dr. Wakker as sometimes occurring in badly affected bulbs.

A repetition of the carrot cultures in 1899 led to similar results.

In two test-tube cultures which were examined after seventy-two days the growth appeared to be typical for *Ps. hyacinthi*, but in one the carrot was browned and in the other not. In both cultures there was a feeble smell, like glue; in both the cylinders were softened and went to pieces under slight pressure of the fingers. In the one which was not browned the carrot was distinctly but feebly acid to neutral litmus paper (it was also acid on the forty-second day). In the other the surface slime was neutral to litmus (it was alkaline on the forty-second day). The interior of the carrot was also neutral or nearly so. Lead acetate-paper placed for six weeks in the mouth of this tube, below the cotton plug, was not browned. The cylinders are believed to have been derived from different carrots. Both cultures were inoculated from the same tube. The difference in brown staining is believed to be attributable to slight differences in the chemical composition of the carrots. (See Ps. phaseoli under The Brown Pigment.)

Sweet Potato.

This medium was prepared in the same way as the common potato. *Ps. hyacinthi* grew well upon it and with little, if any, retardation. Usually, by the end of the first week, at 18° to 25° C., the growth on the aerial part was copious. This growth did not stop early, as in case of the common potato, but continued for a long time, covering the whole of the exposed part and filling up the water with a solid yellow slime. In one set of cultures, at the end of the twenty-second day, the growth was 10 times as abundant as on the common potato, and on the fifty-sixth day 100 times as abundant. In another set of cultures, made some months later, there was "much more growth than takes place on the Irish potato." Usually, by the end of the third week, the 1 to 2 c. c. of water in the bottom of the tube was grown full of the yellow slime, so as not to flow when tilted.

The surface of this growth was wet-shining even in old cultures (55 days). At first the surface was smooth, but after some weeks it became uneven, i. e., thickly set with smooth-roundish prominences, which appearance I have designated as shagreen. This uneven surface remained wet-shining and homogeneous in color, and I have no doubt as to the purity of the culture. Even in cultures not older than one week the bacterial mass did not readily dissolve or shake apart in water.

The color of *Ps. hyacinthi* on this substratum at the end of the first week was wax-yellow to gamboge-yellow in one set of tubes and in another it was "bright-yellow." On the thirty-first day the slime was slightly sticky and its color in mass, on white paper, was maize-yellow. Examined microscopically at this time there were no spores but a great many slender chains (6 to 12 rods) mixed in with zooglœæ and single and paired rods. After 55 days the slime in one set of tubes was "dull-yellow" and in another set "dirty yellow," but there was no distinct brown pigment. At this time, in one set of tubes, the slime consisted of a mixture of long and short rods, chains, and zooglœæ. Some of the rods and chains were very long, extending one-sixth to one-fifth of the way across the field of the microscope (Zeiss 4 mm. apochromatic and 12 compensating ocular). At this time, in the other set of tubes, there were numerous roundish zooglœæ embedded in the bacterial layer. These zooglœæ were a little whiter than the body of the slime and dissolved slowly in water. Under the microscope they presented the same appearance as all the zooglœæ of this organism, and I had no reason to suspect contamination.

An acid appears to be formed out of this substratum. After 31 days the slime from the bottom of a tube showed no alkaline reaction, but was neutral to good neutral litmus paper. After 56 days slime from the same cultures was still "neutral or slightly acid" to litmus, there being no alkaline reaction whatever (2 tubes). After 55 days' growth the slime from another set of tubes showed no trace of alkaline reaction. That from one tube was "neutral or slightly acid" when stirred up in a drop of distilled water and tested with neutral litmus-paper, while that from another tube was "feebly acid." These results may be compared with those obtained from old cultures on common potato.

SUGAR BEET.

The white sugar beet was prepared for use in the same way as the potato cylinders.

Ps. hyacinthi grew copiously on this medium and for a very long time. Usually, at 20° to 25° C., growth was visible by the end of the fourth day, and sooner if very copious inoculations were made; but in some instances growth did not appear until the sixth day, i. e., there was some retardation. Once, on rather dry cylinders 3 months old, the germ refused to grow, although it grew promptly in check tubes of freshly prepared coconut. Moreover, although inoculated very copiously on several different occasions, the organism could not be induced to grow in a flask containing several hundred grams of ground beets covered with 100 c. c. of distilled water. This failure was attributed to the acidity of the beet juice, since the organism grew readily in another flask, which was prepared at the same time and from the same beets, and differed from the preceding flask only in having the first 100 c. c. of water poured off after some hours and another 100 c. c. added.

Generally, by the end of the first week, the whole or nearly the whole of the aerial part of the cylinder was covered, but the early growth was not as copious as in corresponding tubes of *Ps. campestris.* In time this growth became very copious, and the fluid gradually filled up with a solid yellow slime. In 20 days (2 tubes) the growth was "much better" than on potato. In 22 days (another series) the growth was 3 times as much, and in 31 days 20 times as much as on potato. In 37 days (another series) there was a copious growth over the whole cylinder, and the fluid in the bottom (1 to 2 c. c.) was full of the yellow slime, there being at least 50 times as much development as on the potato. Judging from its appearance, this culture continued to grow for another month. After 55 days (another series) there was a much better growth than on potato. The growth in the air was copious, but not all of the fluid was filled with the slime. After 52 days (another series) the beet cylinder was entirely covered with a very copious growth and the fluid around the lower one-half was filled full of a yellow slime, exactly as if it were the cabbage or bean parasite growing on potato. In tubes of potato, inoculated at the same time from the same culture, the organism had made only a feeble to moderate growth and had formed no yellow slime in the water, the contrast being very striking. After 135 days this culture on sugar beet was still fresh-looking, and the solid yellow slime where the water had been was 2 cm. deep.

The surface of this growth was always wet-shining, but sometimes it was smooth and at other times shagreened. Of two cultures examined on the fifth day, the one grown at room temperature was smooth, the one kept in the thermostat was shagreened. Of two other cultures examined on the twenty-second and thirtieth days, both grown at room temperatures, one was smooth and the other was shagreened, i. e., thickly set with smooth, roundish papillæ, which appeared gelatinous to the eye but lifted out readily when touched with the loop. The smooth culture was paler yellow than the other. Portions of the latter did not dissolve readily in water. Under the microscope this culture appeared to be all one thing. In a pale-yellow culture 30 days old there were no spores, but many dense aggregates (zooglœæ) not readily dissolving in water. The rods were short and slender, and no chains or motile elements were visible. In the same culture, after 55 days, there were colonies or zooglœæ in the surface slime. These had roundish margins and were paler yellow than the body of the slime. In a beaker of water they did not dissolve in one-half hour.

At first the cultures were not sticky (8 days), but eventually they became slightly stringy (30 days).

The color of the growth was distinctly yellow from the start, in most cases becoming bright yellow. In mass on white paper, on the eighth day, this color was between gamboge yellow and chrome yellow (Ridgway). After 21 days another culture was gamboge yellow and was several shades brighter than a corresponding culture on potato. After 17 days in the thermostat the color was "dull yellow." One culture remained pale yellow for 57 days. Several others grown at room temperatures were bright yellow after 2 months, and one was noted as still bright yellow at the end of 135 days.

Tubes inoculated from a culture 52 days old took readily, showing that a considerable portion of the culture was living.

An acid seems to be slowly developed in small quantities by the growth of the organism on this substratum. In one tube, at the end of 7 days, there was no acid reaction, the fluid being feebly alkaline to neutral litmus paper. On the eighth day, in a tube from another series, the slime was not alkaline and not acid, but exactly neutral. After 21 days, in a tube from another series, the fluid was feebly alkaline. On the thirtieth day, in cultures of another series, the yellow surface slime was not alkaline, but neutral or slightly acid. The fluid in the bottom of this tube was neutral, but the paper reddened on drying. The fluid, however, from a check tube was also neutral at first, but was equally and plainly acid when dry. After

55 days the yellow slime on the aerial part of the cylinder and the fluid in the bottom of this tube were both acid. There was no trace of any alkaline reaction, but this acidity was feeble, i. e., not much, if any, more pronounced than in the dried-out juice of the check tubes. On the thirtieth day, in another tube of the same series, a mass of germs from the top of the cylinder reacted feebly acid, or at least there was no alkaline reaction on neutral litmus paper. After 55 days a large loop of yellow slime from the same tube showed no alkaline reaction when rubbed on neutral litmus paper, not even when stirred up in a drop of distilled water. At the same time no acid reaction could be detected. The slime was neutral.

No brown stain appeared in any of these cultures (67 days).

See also Feeble diastasic action and Relative nutrient value of carbon compounds for additional notes on growth on solid media.

SENSITIVENESS TO ACIDS.

The failure of *Ps. hyacinthi* to produce any immediate symptoms, even when inserted into the hyacinth-leaf parenchyma by the million, the slow progress of the disease when it finally appeared, and the extent to which growth is restricted to the immediate vicinity of the vascular bundles, have been described in Bulletin No. 26. This behavior of the organism in the host plant, which resembles that of *Ps. campestris* in the turnip and cabbage, led me to suspect it might be very sensitive to acids. To test this supposition the following experiments were made:

Acid Beef Broths.

In all cases the rate of growth in beef broth made neutral to phenolphthalein was assumed as the standard.

(1) The first trials were with stocks 286a, 286b, and 286d. Stock 286a was a 1:2 beef broth, to which no sodium chloride or alkali was added, and the acidity of which was +25 of Fuller's scale. Stock 286b was a portion of the same broth rendered neutral to phenolphthalein (0 of Fuller's scale), by caustic soda. Stock 286d was a portion of 286a boiled down so that it was quite yellow and strongly acid, i. e., +80 of Fuller's scale. Each tube contained 10 c. c. of broth. All were inoculated at the same time from an alkaline beef-broth culture 4 days old, and were kept together in feeble light, at room temperatures of 20° to 24° C.

Result.—The *alkaline* broth (286b) clouded in 26 to 72 hours, according as the infection was made with a large loop or with a tiny drop from the tip of a platinum needle. Stock 286a (feebly acid) clouded in 48 to 168 hours, according to manner of infection (loop or needle). Stock 286d, whichever way inoculated, remained clear until the close of the experiment (49 days).

(2) Stock 300c was an old, partially evaporated flask of 286d brought back to its original volume by adding distilled water. Its acidity was +80; i. e., exactly 80 c. c. of $\frac{N}{1}$ NaOH would have been required to neutralize 1 liter, using phenolphthalein as the indicator. Stock 300b was a portion of 300c diluted with an equal bulk of distilled water, so that its acidity was reduced to +40. Stock 300a consisted of a portion of 300c diluted with twice its bulk of distilled water, the acidity being consequently reduced to about +27. Three tubes of each stock were inoculated from an alkaline beef-broth culture 11 days old. All of the tubes were kept together in feeble, diffused light, in well-plugged tubes of resistant glass, at room temperatures of 20° to 23° C. Two of each set were inoculated with large loops, the third with a tiny drop from the tip of a needle.

Result.—In 300c there was no growth whatever (21 days). In 300b growth was much retarded, the fluid remaining clear for 8 days, and probably for a much longer period. On the twenty-first day, when next examined, the two tubes inoculated by loop were feebly clouded, and showed a moderate amount of yellow precipitate. There were also quite a good many large yellowish flecks (zoogloeæ), on the walls and floating in the fluid. In the tube inoculated by needle the clouding was *very* feeble, there was only a *slight* precipitate, and there were no zoogloeæ. On the twenty-fifth day the fluid in the needle culture was neutral to sensitive neutral litmus paper, while in the loop cultures it had become feebly alkaline. In 300a clouding was visible on the sixth day in the loop cultures, and on the eighth day in the needle culture. Here also growth was retarded, but not so long as in 300b; e. g., on the twenty-first day the tube of 300a, which was inoculated by needle, was about twice as cloudy, and contained ten times as much precipitate as the tubes of 300b, which were inoculated by loop. The organism changed the fluids from acid to alkaline, and in the end (55 days) all of the cultures were much alike.

(3) The last experiment was repeated, more attention being paid to the time of first clouding in 300b. Each tube contained, as usual, 10 c. c. of broth, was tightly plugged, was inoculated with one loop (*oese* 2 mm. in diameter) from an alkaline beef broth culture 12 days old, and was set away in feeble light at room temperatures of 19° to 26° C. (mostly 20° to 21° C. during the first 6 days).

Result.—In 300a clouding was first visible on the sixth day, but was then very feeble. In 300b the fluid remained perfectly clear for 19 days. On the twenty-sixth day, when next examined, it was feebly clouded. In 300c there was never any growth (26 days).

Ps. campestris, *Ps. phaseoli*, and *Bacillus amylovorus* also refused to grow in 300c. On the contrary, *Ps. stewarti*, inoculated from a solid culture, grew in it for a long time and very luxuriantly, although clouding did not appear until the eighth day.

LACTIC ACID.

Schering's diabetine (fructose) in 1 gram doses was added to test tubes containing 10 c. c. portions of standard nutrient agar (acidity + 15.5 of Fuller's scale), on which *Ps. hyacinthi* was known to grow well. This agar was resterilized, slanted, and inoculated by streaking, but no growth could be obtained (58 days). The inoculation was from an agar culture 13 days old, a large loop of the yellow slime of *Ps. hyacinthi* being rubbed thoroughly over the whole surface. That the culture used for inoculation was alive was shown by the fact that an inoculation therefrom into the same agar without the sugar produced a decided growth in 24 hours. This fructose agar was distinctly acid to neutral litmus paper, owing presumably to the presence of a small amount of lactic acid which is said by the manufacturers to be put into the sugar to improve its keeping qualities. Ten grams of this sugar required 10 c. c. of $\frac{N}{10}$ NaOH to render it moderately alkaline to litmus. When 0.7 c. c. and 1.0 c. c. portions of this alkaline sirup were added to tubes of this agar a substratum was obtained on which, after a time, the organism grew luxuriantly. The inoculations were made with a loop of slime from solid cultures. In the 0.7 c. c. tubes, growth was feeble during the first 4 or 5 days, then excellent and long-continued. In the 1.0 c. c. tubes, growth at the end of 7 days was still very feeble, i. e., not one one-hundredth as much as in the tubes containing only seven-tenths as much of the alkaline sugar. On the twelfth day there was about one-third as much growth; on the sixteenth day growth had much increased. After this the 2 sets of tubes looked much alike and the growth was at least 10 times as abundant as on the same agar without the sugar.

POTATO BROTH.

This broth was half strength, i. e., 1:4. It was made by putting 500 grams of thinly sliced potatoes into 1,000 c. c. of distilled water and heating on a water bath 2 hours at 40° to 55° C. The broth was then filtered, steamed one hour, cooled, made up to 2,000 c. c., filtered, tirated, and divided. Its acidity was +30 of Fuller's scale. For comparison, a portion of this broth received enough caustic soda to make it +24, another portion received one-third as much soda and registered an acidity of +28, a third portion received 1 per cent of Witte's peptonum siccum. This last was not titrated, but the peptone is known to give an alkaline reaction with litmus, and this addition must have considerably reduced the acidity of the fluid.

(1) Each tube contained 10 c. c. of broth and was well plugged. All were inoculated at the same time and were kept together in feeble diffused light, at room temperatures ranging from 20° to 25° C. Each tube was inoculated with a large loop from a well clouded alkaline beef broth culture 11 days old.

Result.—The simple potato broth (+30) powerfully retarded the growth of *Ps. hyacinthi*, the fluid remaining perfectly clear for 8 days, and probably much longer. On the twenty-fourth day, when next examined, the fluid was clouded, showed some precipitate and had become alkaline. The +24 and +28 broths were both feebly clouded in 72 hours. The peptone potato broth must have clouded somewhat earlier than the last two, as it showed distinctly more growth at the end of the third day. Query: What was the inhibiting substance represented by the difference between +30 and +28 and removed by the addition of this small amount of sodium hydrate? Could it be oxalic acid?

(2) The preceding experiment was repeated, all of the conditions remaining the same, except that fewer germs were put into the tubes. The inoculations were from an alkaline beef broth culture 12 days old, and each tube received a moderate sized loop instead of a large loop.

Result.—In the +30 broth, which was feebly acid to litmus, no growth ever appeared (31 days). In the +24 broth a very feeble clouding was visible in 68 hours. In the +28 broth clouding was visible in 44 hours. Feeble clouding also appeared within 44 hours in the peptone potato broth.

Ps. campestris and *Ps. phaseoli* also refused to grow in the +30 broth. *Ps. stewarti* grew in it readily.

Malic Acid.

This acid was added to gelatins (see Nutrient gelatins) and to the potato broth already described. A portion of this potato broth was measured out and enough of this substance was added to raise the acidity of the broth from +30 to +45. A tube was inoculated with a large loop of *Ps. hyacinthi* from the same culture used for the first potato-broth experiments. The tube was exposed to the same favorable conditions as the potato-broth tubes, but no growth ever appeared (55 days). A month later the experiment was repeated, inoculating with a moderate sized loop from the culture used for the second potato-broth experiments. This tube was subject to the same conditions as the latter, but no growth ever appeared (31 days). A month later, 2 more tubes were inoculated, using an enormous number of germs, viz, for each tube a mass of bright yellow slime 2 mm. in diameter, which was taken from the fresh surface of a starch-jelly culture 9 days old. These well-plugged tubes were kept in very feeble diffused light, at room temperatures of 23° to 30° C., but no growth ever appeared (80 days).

Ps. campestris and *Ps. phaseoli* also refused to grow in this broth. On the contrary, *Bacillus amylovorus*, inoculated from a colony, clouded it in 48 hours and in the end made a better growth in it than in alkaline beef broth. *Ps. stewarti* grew in this broth without retardation. Three saprophytic bacteria, obtained by Mr. A. F. Woods from the surface of carnation leaves, also clouded this broth in 2 to 7 days, viz, a pink buff germ, a lemon yellow germ, and an orange colored germ, the latter probably identical with *Bacterium dianthi* Arthur and Bolley. (See also Growth in fluid media.)

CABBAGE JUICE.

This fluid was prepared by grinding green cabbage leaves and extracting the juice under pressure. No water was added. The leaves were from old, slow-growing, hothouse plants. This juice was divided into two portions, one of which was sterilized by forcing it through a Chamberland filter, and the other by steaming for a few minutes on 3 consecutive days. There was no difference in the acidity, each titrating +40 with caustic soda and phenolphthalein. The boiled juice smelled strongly of cabbage. Each stock was inoculated in the same way, i. e., with a small mass of bright yellow slime from a starch-jelly culture 28 days old. The tubes were well plugged and set in a dark place exposed to room temperatures of 22° to 33° C. (mostly 25° to 29°).

Result.—One tube of the filtered juice was under observation 44 days, but no growth appeared. Two tubes of the boiled juice were under observation, respectively, 29 and 44 days, but there was no growth. Five tubes of slant agar were inoculated at the same time from the same culture, and all took readily. Knowing that bacteria will tolerate more acid in a solid than in a fluid medium, 150 mgs. of Lautenschläger's neutral agar flour was added to one of the tubes on the twenty-ninth day. This was then steam sterilized, slanted, and the surface carefully streaked with at least a cubic millimeter of bright yellow slime from an agar culture 4 days old. This slant culture was under observation, in conditions favorable to growth, for 45 days, but no growth ensued, except on the wall of the tube above the slant in a place which was accidentally touched by the loop and where a little moisture condensed.

Ps. phaseoli and *Ps. campestris* also refused to grow in this acid cabbage juice; but when the fluid was solidified by adding 150 mgs. of the agar flour the latter made a very copious and prolonged growth—i. e., much better than on ordinary agar, although it was started upon it with great difficulty (3 copious inoculations). On the contrary, *Bacillus amylovorus* and *Ps. stewarti* grew in the boiled juice without retardation. The latter, inoculated from a solid culture, clouded the fluid (2 tubes) in less than 48 hours and made a very prolonged and copious growth. *B. amylovorus* grew nearly as well.

TOMATO JUICES.

Four tomato juices were tried, all from fruits of thrifty hot-house plants of one variety (Lorillard). The fruits were picked and sorted into groups as follows: (1) Stock 331, fruits red and ripe, with a fine odor, excellent for the table; (2) stock 332, fruits full grown and yellowish-green, i. e., commencing to ripen; (3) stock 333, fruits entirely green, but nearly or quite full grown; (4) stock 334, small green fruits, one-twentieth to one-fourth grown. The juices were obtained by pulping the fruits and extracting under pressure. These fluids were then filtered, steamed, filtered, filled into tubes, and sterilized by steaming 10 minutes on 2 consecutive days and 15 minutes on the fourth day. Each juice was carefully titrated for acidity and sugar content. Starch was abundant in the green fruits, but there was very little in the yellowish-green fruits and none whatever in the ripe fruits. Grape sugar was most abundant in the yellowish-green fruits. The acidity of the yellowish-green and of the ripe fruits was nearly the same, but undoubtedly they contained more than one acid, and the proportions were probably different. Each of these stocks was inoculated with at least one-half cubic millimeter of the yellow slime of *Ps. hyacinthi* from a coconut culture 7 days old, a check inoculation (which grew promptly) being made into alkaline beef broth. All of the tubes were kept together in feeble diffused light at room temperatures which ranged from 22° to 34° C. (mostly 25° to 28°) during the first 25 days, and after that 29° to 35° C., and occasionally for a few hours as high as 37° (Washington summer heat). The results obtained are given below:

(1) Stock 331. No growth (35 days). The acidity of the stock was +64, and the sugar content was such that 2.5 c. c. were required to reduce 5 c. c. of the standard solution of $CuSO_4\ 5H_2O$ in Soxhlet's solution.

(2) Stock 332. No growth (35 days). The acidity of this stock was +68, and the sugar content was such that only 1.8 c. c. were required to reduce 5 c. c. of the standard solution of $CuSO_4\ 5H_2O$.

(3) Stock 333. No growth (35 days). The acidity of this stock was +55, and the sugar content was such that 3.7 c. c. were required to reduce 5 c. c. of the standard solution of $CuSO_4\ 5H_2O$.

(4) Stock 334. No growth (35 days). The acidity of this stock was +59, and the sugar content was such that 2.2 c. c. were required to reduce 5 c. c. of the standard solution of $CuSO_4\ 5H_2O$.

The acidity here recorded marks the first perceptible trace of change of color on adding $\frac{N}{10}$NaOH drop by drop to 5 c. c. of the juice in 50 c. c. of water plus 1 c. c. of the standard alcoholic solution of phenolphthalein. More alkali was required to produce a bright pink, and, if this be taken as the standard color, then the readings would be, respectively, +74, +75, +65, and +72. Still more alkali was required to produce a red which could not be made deeper. The readings when addition of more alkali did not deepen the color were, respectively, +111, +88, +81, and +93.

In each case these figures are the average of three titrations at room temperatures. Titrated boiling hot, each of the fluids required considerably *more* alkali.

Ps. campestris, *Ps. phaseoli*, *Bacillus amylovorus*, and *B. oleæ* also refused to grow in these juices. On the contrary, stocks 333 and 334 were well clouded by *Ps. stewarti* on the fifth day, and 331 became well clouded some time between the eighth and fifteenth day. In each of these 3 fluids this organism made a copious and prolonged growth, but it refused to grow in 332, although this contained more sugar than the other stocks; and even when reinoculated copiously from 331, after the latter had become well clouded, it remained clear. It is probable, therefore, that the limit of toleration of *Ps. stewarti* for the acids of the tomato lies between +64 and +68.

HYACINTH BROTH.

Perhaps the most interesting result of all was obtained with hyacinth broth. This was made from 13 rather small bulbs of a single-flowered white variety of *Hyacinthus orientalis*. The bulbs had been kept in a closet in the laboratory all winter and had lost some water, but were not shriveled. In March the plateaus were removed (the most alkaline part of the bulbs) and the remainders were pulped and an effort made to extract the juice. Only a very sticky slime oozed through the bag, and this would not flow. I then added 100 c. c. of distilled water and squeezed out as much juice as possible under an iron press. An endeavor was made to pass the fluid through a Chamberland filter, but it would not go through with a pressure of 25 pounds per square inch. The fluid was then thoroughly steamed and filled into test tubes after filtering out a very copious white coagulum, consisting principally of nitrogenous substances, starch, and raphides. There resulted a hazy, yellow, acid fluid, which never precipitated entirely clear. Titrated with caustic soda and phenolphthalein, 1 c. c. exactly balanced 0.28 c. c. of $\frac{N}{10}$NaOH (first trace of color), and consequently the acidity was +28 of Fuller's scale. Pushed far enough to give a bright pink, the reading was +40. This fluid was moderately acid to neutral litmus paper. Four clean, well-plugged tubes, each containing 10 c. c. of this fluid, were inoculated with a large loop of *Ps. hyacinthi* from an alkaline beef-broth culture 2 days old, which broth was inoculated from a solid culture and had been cloudy for 24 hours. The tubes were kept in a dark place at room temperatures very suitable for growth, viz, 19° to 25° C.

Result.—This fluid exerted a profound restraining influence. In two of the tubes the first bacterial clouding appeared on the seventeenth day, at which time there was no rim of germs, pellicle, or precipitate. The other 2 tubes were not clouded on the seventeenth day, but on the thirty-seventh day, when next examined, there was a copious growth in each. A large loop taken from each of these 2 tubes on the eighth day and put into tubes of alkaline beef broth did not cloud the latter until the fifth day, from which we may infer that multiplication had gone on in the acid broth very slowly. When once the restraining influence was overcome, the organism ran riot in the fluid making a magnificent and long continued growth, more growth in fact than I had been able to obtain with any other fluid. On the thirty-seventh day the fluid in each tube was plainly alkaline to litmus; there was no pellicle but a dense bright yellow rim 4 mm. wide, and a yellow precipitate 5 to 6 mm. deep. The rim was homogeneous, i. e., not composed of scattered yellow zoogloeæ on a paler film, as was, however, the rim in tubes of alkaline beef broth inoculated directly from these cultures. This rim was wrinkled, or traversed crosswise, by many denser bands. The color of the bacteria was as bright as in the vessels of the host plant; compared with Ridgway's tables it exactly matched his chrome yellow (VI–8). On the fifty-second day all the tubes were alike. Each had a thick dark yellow ring above the fluid, and a copious, distinctly yellow pellicle. The fluid was nearly clear and distinctly pale brown, which was not the case with the broth in the uninoculated tubes. The yellow precipitate was three times as abundant as that obtained in alkaline beef broth, i. e., 6 to 7 mm. deep. The fluid was now strongly alkaline, and the germs were somewhat ropy. The cultures had a feeble, fishy odor suggestive of amin compounds. On boiling, gases were given off which immediately and strongly blued neutral litmus paper. Conducted into a tube of Nessler's solution, the vapor from the boiling fluid caused an immediate copious rusty precipitate. The same result was obtained by putting one-fourth c.c. of the filtered fluid into Nessler's solution, but no such reaction could be obtained from the uninoculated fluid. An attempt was made to determine the amount of alkalies present and the results are given, but I am not confident that either one is of any value. The fluid did not redden with a small quantity of phenolphthalein, but reacted with a larger quantity. Titrated in ice water with 6 c. c. of the standard solution of phenolphthalein, 3 c. c. of the fluid required 0.20 c. c. of $\frac{N}{10}$ HCl; titrated with neutral litmus, 3 c. c. required 0. 30 c. c. of $\frac{N}{10}$ HCl. It was difficult to drive off all the volatile alkalies by boiling, the blue reaction on wet litmus paper showing plainly in the steam when the fluid was half boiled away.

This experiment with hyacinth broth was repeated, inoculating a tube of the same stock with a moderate-sized loop from an alkaline beef-broth culture 12 days old, i. e., with more germs. All the other conditions were the same.

Result.—The first trace of growth was on the fifteenth day. On the nineteenth day there were distinct rolling clouds and a yellow rim, but no precipitate. Subsequently there was a copious growth and a very heavy precipitate. No crystals formed.

Ps. phaseoli and *Ps. campestris* both grew in this fluid, the latter much more readily than the hyacinth germ.

The discovery of this sensitiveness to acids furnished a satisfactory explanation of some perplexing contradictions obtained with unneutralized beef, potato, and cauliflower broths early in my study of *Ps. hyacinthi*. It also afforded a partial explanation of the slow progress of the disease in the host plant, but apparently not a full one, since once well established in the vessels, it is not clear why the parasite does not immediately advance into and destroy the acid parenchyma under cover of the alkalies which it produces. Evidently there are additional difficulties to be overcome, one of which will be discussed in the following section.

FEEBLE DIASTASIC ACTION.

The meager development on cooked potato led to the belief that something in this substratum inhibited the growth of the hyacinth organism. In the beginning it was thought that the feeble growth might be confined to certain varieties of potatoes and that on others a better growth could be obtained. To test this, cultures were made on a variety of tubers, new and old, but with the same result. Subsequently tubers were procured from a variety of soils and from climates as different as New York and Florida, but there was little difference in the amount of growth. The growth was comparatively feeble no matter what the age or origin of the potato. It was then thought that possibly the acidity of the potato might be the restraining cause, and dilute caustic soda was added to potato cylinders, so as to render them neutral or feebly alkaline after they were steamed. On such cylinders the organism grew little if any better than on the untreated potatoes (41 days), and this hypothesis was also abandoned. I then began to suspect that the feeble growth was wholly a matter of insufficient nutrition, and found that on adding considerable quantities of cane sugar the growth increased rapidly and became very abundant. About the same time tests with iodine showed that the starch of the potato, even close under the bacterial layer, had been very little acted upon by the organism.

The rather meager growth of this germ on potato now appears to me attributable to its feeble diastasic action, i. e., to its inability to get from the starch enough food for its normal growth, and I am surprised that this explanation did not occur to me at once. The organism grows fairly well until the small amount of grape sugar present in the potato is exhausted, and thereafter, when thrown wholly upon its own resources, makes only an extremely feeble growth, corresponding to its very feeble diastasic powers. This conclusion rests upon the following experiments:

IODINE STARCH REACTION.

My uninoculated potato cylinders when tested with iodine potassium iodide diluted with water, or with iodine crystals dissolved in absolute alcohol to saturation and then diluted with water as required for use, always yielded an immediate bright blue reaction. The starch reaction was also strong after the *Ps. hyacinthi* had been grown on them for several weeks, although there was always evidence of slight diastasic action to be found in the purplish color assumed by some of the grains. The following are transcripts from my notes.

(1) Some fragments of potato scraped from immediately under the yellow slime on a culture 30 days old were put into an old solution of iodine-glycerine. They became black at once, and when crushed out and examined under the microscope were brownish purple--i. e., more brown purple than the starch from a check tube. Tested with alcohol-iodine diluted with fifteen or twenty times its bulk of water, the starch of the potato in the check tubes became pure blue. In the culture, immediately under the yellow slime, most of the starch-bearing cells became purple, but occasionally one was nearly pure blue. Cells deep in the cylinder reacted blue.

(2) On the thirty-first day another tube of the same lot was tested with alcohol-iodine, diluted with thirty or forty times its bulk of water. When this fluid was put on scrapings from a check tube, the reaction was pure blue; when it was put on scrapings from immediately under the yellow slime, the starch reaction was purple and blue purple.

(3) A year previous scrapings were made close under the bacterial layer of a culture 36 days old and tested with iodine potassium iodide. There was a strong blue-black reaction. Under the microscope, however, some of the cells were paler than others, indicating that some of the starch grains had been acted upon slightly.

(4) On the twenty-ninth day a potato cylinder, bearing a typical growth of the yellow slime and uniformly grayed, was broken across the middle and tested with iodine alcohol in water. The middle part of the cylinder reacted blue. The outer part, close under the bacterial layer, gave either a reddish or purplish blue reaction.

No potato cultures of this organism were ever tested which did not give a very decided reaction with iodine. The importance of this fact will be brought out to best advantage by comparison with *Ps. campestris* or *Ps. phaseoli*, both of which exert on starch a very powerful diastasic action. When either of these germs is grown on potato cylinders in water for 30 days, not simply all of the starch in the surface cells, but also all of that in the deeper parts of the cylinder, is acted upon, and this action is not feeble, but so vigorous and far-reaching that if the whole cylinder is crushed in a large bulk of the iodine water there is either no color reaction whatever, or merely in places a feeble brownish-purple tinge, indicating that all of the starch, or almost all, has been converted.

Growth on Potato with Addition of Cane Sugar.

These cylinders were the ordinary potato cultures in test tubes, to each of which was added 1 gram of cane sugar. At first growth was retarded—e. g., on the fourth day it was slight and white or nearly white. On the thirty-seventh day it was yellow, extended down into the fluid, and was 20 to 25 times as abundant as in the check tubes. The surface was wet-shining, but not smooth, owing to the protrusion of rounded zooglœæ. On the sixty-seventh day the slime was wax yellow, and covered the whole cylinder, just as *Ps. campestris* or *Ps. phaseoli* would have done without the addition of sugar. The entire culture now looked like shagreen from inequalities in its surface due to the protrusion of rounded masses. The slime was neutral, or at least not alkaline, and the small amount of fluid remaining in the bottom of the tubes was plainly acid to neutral litmus paper. The brown stain of the fluid was less than in the check tubes.

Growth on Potato with Addition of Maltose and Dextrine.

This medium consisted of potato cylinders standing, two-thirds covered with distilled water, in well-plugged test tubes. To each tube was added 100 milligrams of maltose and an equal quantity of dextrine. They were then re-steamed as usual (20 minutes at 100° C., on 3 consecutive days), constituting stock 301. Each tube was inoculated with a large loop of *Ps. hyacinthi* from a well-clouded beef-broth culture 11 days old, check tubes made from the same tuber being held for comparison.

Result.—During the first few days (4 at least) there was not as much growth in the 2 maltose-dextrine tubes as in the 2 check tubes. However, at the end of 24 days (temperature 19° to 25° C.) there was an abundant yellow, wet-shining growth over the whole of the exposed part of the cylinder, down into the upper part of the water, and on the wall of the tube, at least 15 times as much growth as in the check tubes. This growth continued for several weeks.

GROWTH ON POTATO WITH ADDITION OF DIASTASE OF MALT.

This medium consisted of 4 potato cylinders from the same tuber as 301, to each of which was added 500 milligrams of Merck's "diastase of malt absolute." After remaining over night in a water bath at 50° C., these tubes were sterilized by steaming about 20 minutes on 4 consecutive days. Each tube received a large loop from a beef-broth culture 11 days old—the same tube that was used to inoculate the tubes of potato-maltose-dextrine. Two tubes without the disastase were inoculated for comparison. The tubes were kept together in the dark at room temperatures of 20° to 25° C.

Result.—By the end of the third day the check tubes had developed a thin, distinct, yellow growth over nearly the whole of the exposed part (one-third) of the cylinder. The progress of these check cultures from this time on was typical for *Ps. hyacinthi*, there never being any copious growth or any development of the yellow slime under the water. The tubes to which the diastase was added were under observation 55 days. In 3 of them there was never any growth. In the fourth tube growth was retarded until the eighth day (temperatures 20° to 23° C.), on which date a yellow patch 1 cm. square was visible. On the twenty-fourth day the organism had entirely overcome the retarding action of the medium and had made an abundant, distinctly yellow, smooth, wet-shining growth over the whole cylinder down into the water and up on the wall of the tube. This growth was estimated at 50 times that in the check tubes and was greatly in excess of any growth ever before obtained upon potato.

The 3 tubes in which there had been no growth were reinoculated on the twenty-fourth day, using for one a large loop of yellow slime from one of the check tubes, and for each of the other two an equally large loop of slime from the other check tube. This slime was rubbed carefully over the surface. No growth ensued, although the tubes were watched for a month. The fluid in these tubes was neutral to litmus, or very feebly acid when dry, and the restraining influence was therefore attributed to an excess of maltose or dextrine liberated by the diastase. On mashing one of these cylinders in alcohol-iodine water there was no starch reaction whatever nor any red reaction.

POTATO STARCH IN PEPTONE WATER WITH DIASTASE.

This medium was prepared in test tubes of resistant glass, using about 1 gram (estimated dry weight) of freshly prepared thoroughly washed potato starch to about 9 c. c. of distilled water which had received 4 per cent of Witte's peptonum siccum. The tubes were then put into the steamer and the starch solidified in a slanting position. Some of these tubes were held as checks, and the surface of the remainder was flooded with about 1 c. c. of distilled water containing the commercial Taka-diastase. Each tube received 20 milligrams of this diastase, which was allowed to act 4½ hours at 23° C. and then destroyed by steam heat. These tubes were then inoculated from three different cultures of *Ps. hyacinthi*, a beef-broth culture 14 days old, a turnip culture 9 days old, and a carrot culture 9 days old. The fluid loops were streaked; the solid loops were rubbed carefully over the whole slant. The tubes were then kept in the dark at room temperatures ranging from 19° to 23° C.

Result.—In the check tubes at the end of 48 hours there was a slight to very slight growth. On the eighteenth day from one-fourth to three-fourths of the slant surface in these tubes bore a thin bright yellow growth, which never increased much. The development of the germ in the tubes which received the diastase was plainly different. At the end of 48 hours the growth was distinctly yellow and much better than in the check tubes. On the ninth day the principal difference was still the amount of growth which was several times that in the check tubes. On the eighteenth day the growth was dirty yellow, wet-shining, and copious, i. e., at least 10 times as much as in the check tubes. The difference in color was very decided. The slime in the check tubes was pure yellow; that in the others was dirty yellow, verging into brownish. The tubes were now thought to be rather too dry, and 2 c. c. of sterile Potomac River water was pipetted into each one, the result being a somewhat increased growth. On the forty-fourth day the growth in the check tubes was still feeble and much less than in the tubes which received the diastase. The substratum of the latter had become brownish-white with the merest trace of pink in it. The same stain appeared in the check tubes, but was much feebler.

Tubes of *Ps. campestris* and *Ps. phaseoli* yielded some instructive comparisons. In the check cultures, on the sixteenth day, the growth of these two germs was at least 20 times as abundant as that of *Ps. hyacinthi*. On the seventy-third day, in the check tubes, the layer of *Ps. hyacinthi* was still feeble, and was still distinctly yellow; that of *Ps. campestris* and *Ps. phaseoli* was 100 times as abundant and had lost all of its pure yellow color, this having changed into a decided brown. The starch in the check tubes of the hyacinth germ was as firm, elastic, and insoluble as when first inoculated, and was but little stained; that in the corresponding tubes of *Ps. campestris* and *Ps. phaseoli* was gray, soft-mushy, and soluble in water. Tested in alcohol-iodine diluted with 50 volumes of distilled water, the check cultures of *Ps. hyacinthi* gave a strong starch reaction; those of *Ps. campestris* and *Ps. phaseoli* gave no color reaction whatever. One culture of each was also tested with Soxhlet's solution for the presence of reducing substances. *Ps. campestris* and *Ps. phaseoli* each reduced 25 c. c. of the standard solution of copper sulphate (34.639 grams of c. p. $CuSO_4$ 5 H_2O

in 500 c. c. of H_2O). The tests were made in the usual way with 5 c. c. portions of the copper solution and 5 c. c. of the alkaline solution in 40 c. c. of distilled water, boiling 2 minutes in white porcelain capsules. The check culture of *Ps. hyacinthi* was estimated to have reduced only a small fraction of 1 c. c. of the copper solution. On settling there was only a little red precipitate and the fluid was still quite green-blue, so that perhaps not more than one one-thousandth of the starch was converted. The cabbage and the bean germ grew as well, or very nearly as well, on the peptone-potato starch without the diastase as with it, the cultures looking much alike.

Inasmuch as the Taka-diastase contained a trace of some reducing substance and the peptone in water was able of itself to nourish the hyacinth germ for a time, it was thought best to repeat this experiment, using solutions of mineral salts, sodium asparaginate and ammonium lactate in place of the peptone, the same kind of starch, and a Taka-diastase, reprecipitated for me by Dr. John M. Francis.

NUTRIENT STARCH JELLY NO. 1.

This medium was prepared from Uschinsky's solution, substituting potato starch for the glycerine. My method of preparing this medium and the following one need not be given here, as it has been published in Proceedings of the American Association for the Advancement of Science, Boston meeting, 1898, page 411, and in Centralblatt für Bakteriologie, 2. Abt., Bd. V, page 102. All that is necessary to say is that each tube contained from 5 to 7 c. c. of the solution and 1 to 1.5 grams of the dry starch. After the starch had set and was ready for use the check tubes were counted out and the slant surface of the jelly in each of the others was flooded with 1 c. c. of distilled water containing exactly 20 milligrams of the diastase. These tubes were then put into the thermostat at 34° C. for 1½ hours, and afterwards the diastase was destroyed and the tubes sterilized in the usual way, i. e., by steaming for a few minutes on 3 consecutive days.

Before using, the diastase was carefully tested for the presence of reducing substances and found to be entirely free. This diastase likewise gives no blue reaction with guaiac resin and hydrogen peroxide. The starch jelly was also tested in the same way, using Soxhlet's solution, and was found to be entirely free from reducing substances. On the contrary, bits of starch jelly from the tubes which had been treated with the diastase gave an immediate rusty precipitate when dropped into the boiling fluid.

Three tubes of this medium were inoculated, along with 3 check tubes. These 6 tubes were divivided into 3 lots, each group being inoculated from a separate culture. All were kept in the dark at room temperatures, which ranged from 19° to 25° C. during the first 2 weeks and then from 25° to 34° C. (mostly 25° to 29°).

Result.—Two of these groups of tubes failed to catch and were reinoculated later so that the first group will be considered by itself.

(1) These 2 tubes were inoculated in the same way from a fluid culture 32 days old. During the first 18 days there was no trace of color or sign of growth in the check tube. On the twenty-seventh day there was a slight growth with feeble yellowing of the surface, but careful scrutiny was necessary to detect it. On the thirty-fifth day a slight increase of growth was noted. The starch had not dried out much and the whole of it was still bluish white, indicating that there had been no considerable diastasic action. The streak was very thin, very pale yellow, did not hide the substratum, and had no well-defined margins. On the sixty-second day there was decidedly more growth, the whole surface being covered with a thin, distinctly yellow, smooth, homogeneous, wet-shining layer. The body of the starch still preserved its bluish white luster and retained its water well. The amount of growth in this tube after 62 days was not greater than that present in the other tube at the end of 5 days. In the tube which received the diastase there was, on the fifth day, a distinct but not very copious growth, covering about two-thirds of the slant surface. On the twelfth day there was an abundant bright yellow growth covering the whole surface and affording a striking contrast to the check tube. This contrast continued for some time, the difference in the 2 tubes on the fourteenth day being shown in figs. 15 and 16 of the plate accompanying bulletin 26 of this Division. The color was approximately Ridgway's canary yellow (VI–12). On the twenty-seventh day the slime was still bright yellow, and the amount of growth was estimated at 200 times that in the check tube. On the thirty-ninth day there was still no brown stain.

(2) After 8 days the other 4 tubes were reinoculated copiously over the whole surface with yellow slime taken from the culture just described. They were under the same conditions as to light and temperature, the greatest difference between these and the preceding being the enormous number of germs used in making the inoculation.

Result.—The 2 check tubes behaved alike. On the fourth day there was a trace of yellow growth at the bottom of the slant, but it was feeble, and was visible on not more than one-fiftieth of the whole surface. At this time the tubes which received the diastase showed an abundant bright yellow growth over the whole surface, a growth several hundred times as abundant as that in the check tubes. On the sixteenth day, in the check tubes, there was only a feeble growth of 9 or 10 square millimeters. This growth was bright yellow, but it was not one one-hundredth as much as in the tubes which received the diastase. On the twenty-seventh day, in the check tubes, the growth had doubled, but the substratum was hidden only over a few square millimeters, and the ratio of growth in the 2 sets of tubes was still about the same, viz, 1:100. The starch was still bluish white. On the twenty-seventh day, in the tubes which received the diastase, the growth covered the whole surface of the slant (800 to 900 sq. mm.) with a smooth, homogeneous, wet-shining, canary yellow layer, which was abundant enough to hide the substratum. There was a trace of pink in the starch, but no brown stain. On the thirty-fifth day the starch jelly was removed from one of the check tubes. It was as firm and elastic as when first prepared. On breaking it into fragments and throwing it into boiling Soxhlet's solution (5 c. c. standard $CuSO_4$ 5 H_2O solution; 5 c. c. standard alkaline solution; 40 c. c. distilled water) and continuing the boiling 3 minutes, the fluid was as blue as at the beginning, and the only precipitate of copper oxide was an extremely slight one restricted to those fragments of the jelly which were immediately under the bacterial layer. Certainly not more than one one-thousandth of the starch was converted.

(3) The experiment just described was repeated 3 months later in the warmer weather of midsummer. A new stock of the medium was prepared and in this case each tube received 2 gr. of the dry potato starch and 8 c. c. of the nutrient mineral solution. Instead, however, of converting the starch with diastase, the carbon food was supplied by the addition of various sugars, alcohols, and gums. No mention will be made here of anything but the check tube and a tube of the same stock fortified by the addition of 500 mg. of a dextrine, which contained a substance reducing Soxhlet's solution but no amylodextrine and no substance reducing Barfoed's reagent. Both tubes were inoculated at the same time and in the same way; i. e., each with a large loop of yellow slime from a fructose-agar culture 17 days old, but still in excellent condition owing to its having grown slowly on the start. The tubes were kept in a dark closet at room temperatures ranging from 25° to 32° C. (30° to 32° during the first 5 days). Tubes of *Ps. campestris* and *Ps. phaseoli* were also inoculated at the same time and kept under the same conditions.

Result.—In the check tube of *Ps. hyacinthi* there was no visible growth during the first 48 hours. On the third day there was a very slight growth (barely visible), and the bluish white translucent appearance of the starch remained unchanged. In the tube which received the dextrine, growth was visible in 48 hours, but it was still feeble on the third day; i. e., growth was retarded. On the third day, in the check tubes of *Ps. campestris* and *Ps. phaseoli*, there was 20 times as much growth, and the starch jelly under the slime, to a depth of 2 mm., was changed to a dead, opaque white. Returning to the hyacinth germ, there was on the seventh day, in the check tube, a very thin, pale yellow streak or film down the middle of the slant. In the tube which received the dextrine the whole surface was covered by a thin distinctly yellow layer; i. e., there was several times as much growth as in the check tube, but there was no visible diastasic action. The growth of *Ps. phaseoli* on the check was now at least 100 times as abundant as that of *Ps. hyacinthi* on the same medium.

On the starch jelly with addition of the dextrin *Ps. campestris* and *Ps. phaseoli* both made a good growth. On the seventh day *Ps. campestris* covered the whole surface of the long slant to a depth of 1 to 3 millimeters with a semifluid, smooth, wet-shining slime, and the diastasic action now involved nine-tenths of the starch. The conversion of the starch was clearly visible, proceeding slowly and uniformly from the surface of the slant inward. There was a distinct line of demarcation between the converted and unconverted starch. The latter was bluish white, opalescent, translucent, firm, elastic, insoluble; the former was dead white, opaque, soft, inelastic, and soluble in water on gentle shaking. This part gave no color reaction whatever on adding iodine water. On washing it all out the unchanged one-tenth in the bottom of the tube was seen to have preserved the shape of the original slant, and on adding the iodine water it became bright blue. In the corresponding tube of *Ps. phaseoli* the growth at this time appeared to be equally as good, but only about two-thirds of the starch was converted. The diastasic action proceeded from the surface inward in the same regular manner, the line of demarcation between converted and unconverted starch was equally sharp, and the converted portion had all the peculiarities recorded for that acted on by *Ps. campestris*. A fragment of this soft white starch as big as two peas was stirred up in 5 c. c. of the very sensitive pale brown alcoholic iodine water, but no color reaction could be obtained. This changed starch included all of the outer 5 or 6 millimeters of the slant; on filling the tube part full of water and shaking gently all of it dissolved readily, leaving in the bottom a translucent, bluish white, insoluble, miniature slant, which immediately reacted bright blue on pouring in the same iodine water. These experiments show that the presence of albuminoids is not necessary for the production of the diastasic ferment and also that it is excreted by these two species in the presence of an abundance of readily assimilable food.

On the twelfth day, in the check tube of *Ps. hyacinthi*, the thin, pale yellow growth had extended over most of the slant surface, but it was still not one-hundredth part as abundant as in the corresponding tube of *Ps. phaseoli*, and there was no evidence of any diastasic action, whereas in the latter more than nineteen-twentieths of the starch had been digested. In the tube which received the dextrin, *Ps. hyacinthi* had made, on this date, a good, bright yellow but rather dry growth over the whole surface. On the thirtieth day, in this same medium, there was a plentiful, smooth, wet-shining, bright yellow slime over the whole surface, i. e., growth enough to hide the substratum, but no brown stain, no decided smell, and no ocular evidence of any diastasic action. The germs were carefully scraped off and iodine water poured into the tube, whereupon there was an immediate and general blue reaction, showing that very little of the starch had been changed. This shows clearly that increased growth does not necessarily imply any increased secretion of the diastasic ferment. The check tube could not be compared owing to a contamination.

(4) A few days later another check tube was inoculated and a similar feeble growth ensued. A tube containing 500 milligrams of dextrin, which was inoculated for comparison, gave a much better growth. On the third day the whole surface of the slant in this tube was covered by a thin, distinctly yellow, dry layer, and there was no visible diastasic action. On the twelfth day the growth was smooth, wet-shining, bright yellow, and about 8 times as abundant as in the check tube. There was also a decided diastasic action, involving the outer 5 millimeters of the starch. This result contradicts the preceding experiment with dextrin and is probably attributable to the action of some undetected, intruding organism (see p. 64).

NUTRIENT STARCH JELLY NO. 2.

The nutrient solution used in preparing this medium differed from the preceding by addition of sodium sulphate; by a considerable reduction of the magnesium sulphate and calcium chlorid; by a slight reduction of the sodium chlorid, sodium asparaginate, and ammonium lactate, and by a slight increase of the dipotassium phosphate (for exact composition see *loco cit.*). Each tube received exactly 10 c. c. of this solution and 2 grams of dry potato starch free from any trace of sugar. For comparison a culture was laid at the same time on starch jelly No. 1, containing 2 grams of the same starch and 10 c. c. of the glycerin-free Uschinsky. The slant surface of each substratum was inoculated in the same way, carefully and very copiously, with bright yellow slime from a starch-jelly culture 17 days old. The tubes were kept in a dark place at room temperatures ranging from 21° to 31° C. (most of the time below 28°).

Result.—On the fifth day there was a feeble, bright yellow growth, much alike in each tube, and no visible diastasic action. On the eleventh day there was a thin, bright yellow growth over nearly the whole surface—i. e., a considerable increase of growth, but still no diastasic action. Both tubes were much alike, but there appeared to be slightly more growth in starch jelly No. 1. On the twenty-fourth day the growth in starch jelly No. 2 had increased but little. This growth was wet-shining and distinctly yellow, but so feeble that the substratum was not hidden; there was no brown stain in the substratum, and no visible diastasic action. In starch jelly No. 1 there was distinctly more growth, but no visible diastasic action. An intruding colony (the product of a spore which passed through the sterilizing oven uninjured) had come to the surface, and I suspected that sugar liberated by this colony had diffused through the substratum and stimulated the growth of *Ps. hyacinthi*. On the thirty-fifth day there was no increased growth and no visible diastasic action in jelly No. 2, but in jelly No. 1 the bright yellow growth was 3 or 4 times as abundant and was now clearly attributable to diffusion of sugar, or some other assimilable substance, liberated by the intruding organism. There was no visible diastasic action except in the starch immediately around where this white colony had come to the surface. The effect of the growth of this intruder was most clear cut and interesting.

For comparison with these two tubes a culture was laid at the same time on starch jelly No. 2 with addition of 500 milligrams of dextrin. The organism grew well on this substratum, making 4 to 6 times as much growth as in the check tubes. On the twenty-third day, when last examined, there was an excellent growth and had been for 3 weeks, but there was no visible diastasic action.

Ps. phaseoli was very pale and made a much less abundant growth on nutrient starch jelly No. 2 (made with the modified Uschinsky's solution minus the glycerin) than it did on potato, or than did *Ps. campestris*. In Uschinsky's solution, on the contrary, it was yellower and grew rather better than *Ps. campestris*.

HYACINTH STARCH JELLY.

This was made by adding 1 gram of dry sugar-free hyacinth starch, obtained from bulbs, to 5 c. c. portions of Uschinsky's solution. Three tubes were prepared, to one of which was added 500 milligrams of cane sugar. The tubes were steamed 2 hours on each of 3 consecutive days at 91° C., this low temperature being obtained by putting the tubes in the top of the steamer with the vents left open. The tubes were inoculated with *Ps. hyacinthi* very copiously in the same manner soon after the third steaming from a starch jelly culture 7 days old. They were kept together in a dark place at room temperatures ranging from 15° to 26° C.

Result.—At the end of 48 hours (temperature, 21° to 22° C.) growth was visible in each tube. At the end of the fourth day the 2 check tubes were alike, the whole surface of the long slant being covered with a very thin, distinctly yellow growth. There was, however, no visible diastasic action, the organism behaving on hyacinth starch exactly as on potato starch. In the tube which received the cane sugar there was 4 or 5 times as much growth as in either of the check tubes. This growth was bright yellow and covered nearly the whole surface of the slant, but there was no visible diastasic action, the increased growth being due to the presence of the cane sugar. A little later this tube was accidentally broken. The check tubes were under observation for an additional 24 days, during which time a great change took place in one of them, the growth increasing tenfold. This increased growth of the organism was due to no diastasic action of its own, but to the diffusion of maltose or dextrin liberated from the starch by some buried, slow-growing, white, starch-converting colonies, which originated from spores that found their way into the starch during its preparation and which passed through the steamings uninjured.

The foregoing conclusion is also supported by the fact, already set forth, that *Ps. hyacinthi* grows well on a variety of crude vegetable substances rich in sugar. That this feeble diastasic action partially accounts for the feeble parasitism admits of little doubt. Probably its feeble cytohydrolytic action and its strict aerobism are also restraining influences.

AEROBISM.

As already noted, the buried colonies of *Ps. hyacinthi* in plate cultures grew slowly, and those deepest in the layer of agar or gelatin remained smallest. In the stab cultures also the bacteria gradually faded out in the depths, making much the best growth near the surface. The additional results bearing on the inability of this germ to grow in the absence of free oxygen are thrown together in the following paragraphs.

Fermentation Tubes.

The form of tube used in my laboratory is that devised by Dr. Theobald Smith and made by E. Greiner, of New York.[1] This, by reason of its size and shape, the writer has found more satisfactory than several other sorts he has tried.

First is a table, which sets forth the results obtained with fermentation tubes in 1897. The tubes were filled with distilled water containing 1 per cent of Witte's peptonum siccum and 1 per cent of the sugar or other substance to be tested.

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[1] The fermentation tube, with special reference to anaerobiosis and gas production among bacteria. The Wilder Quarter Century Book, Ithaca, N. Y., 1893, p. 187.

TABLE IV.—*Results of growth of Ps. hyacinthi in fermentation tubes. Inoculated Feb. 12.*

Substances tested in the fermentation tubes in distilled water.		Results obtained.					Duration of experiment. (Days.)
		Clouding.		Gas production.	Reaction to neutral litmus paper.		
Nitrogen.	Carbon.	Open end and outer two-thirds or three-fourths of the U.	Closed end.		Acid.	Alkaline.	
Peptone (1 per cent Wittes's peptonum siccum).	Grape sugar (1 per cent Merck's c. p. anhydrous).	Feb. 15 (third day). Well clouded; hundreds of tiny flecks (zooglœae), which show a decided preference for the upper layers of the liquid, but settle on jarring gently. Feb. 19. Zooglœae continue. Feb. 23. Sharp line of demarcation in the U (three-fourths clouded). Mar. 1. Moderately cloudy; no pellicle; slight precipitate. Mar. 12. Moderately cloudy; no pellicle; considerable pale yellow precipitate. Mar. 20. No change.	Perfectly clear	No gas	No acid.	Slowly increasing alkalinity.	38
Do	Fruit sugar (1 per cent Schering's diabetine).	Feb. 15 (third day), 19 and 23. In all respects like the preceding. Mar. 1. Like the preceding, but a trifle more precipitate. Mar. 12. Moderately cloudy; clouding involves outer three-fourths of U, with sharp line of demarcation; no pellicle; considerable pale yellow precipitate. Mar. 20. No change.	do	do	do	do	Do.
Do	Cane sugar (1 per cent white, granular, commercial).	Feb. 15 (third day). Like preceding. Feb. 19. Same, except no zooglœae visible. Feb. 23. Sharp line of demarcation in the U. Mar. 1. About as cloudy as preceding; a slight precipitate; sharp line of demarcation continues. Mar. 12. Exactly like the two preceding. Mar. 20. No change.	do	do	do	do	Do.
Do	Milk sugar (1 per cent c. p. Once recrystallized in laboratory Div. Chem., U. S. Dept. Ag.).	Feb. 15 (third day) and Feb. 19. Same as tube of grape sugar. Feb. 23. Sharp line of demarcation in the U. Mar. 1. Like tube of grape sugar. Mar. 12. There is now noticeably less precipitate than in the tubes containing the grape, fruit, or cane sugar; otherwise the same. Mar. 20. No change.	do	do	do	do	Do.
Do	Maltose (1 per cent c. p. from Div. Chem., U. S. Dept. Ag. Tested with polariscope and found to give proper rotation.)	Feb. 15 (third day). Like the tube of grape sugar. Feb. 19. Like the tube of grape sugar. Feb. 23. Sharp line of demarcation in the U. Mar. 12. Moderately cloudy; no pellicle; slight precipitate; precipitate plainly less than in tubes containing the grape, fruit, and cane sugar; much like that in tube containing the milk sugar. Mar. 20. No change.	Feb. 19. Clear. Feb. 23 Very feebly clouded, perhaps one-twentieth or one-thirtieth as much as in open end. Clear, except on very close inspection. Possibly the clouding may be due to some accidental tilting of the tube.	do	do	do	Do.

			Mar. 1. No change; doubtful if any growth. Mar. 12. Perfectly clear.				
Do.	Dextrin (1 per cent commercial 10 times precipitated with alcohol in laboratory of Div. Chem., U. S. Dept. Agr. This dextrin contained a substance reducing Soxhlet's solution, but did not redden with iodine and did not reduce Barfoed's solution.)	Feb. 15 (third day), Like the tube of grape sugar except that the zoogl[oe]ae can not be made out clearly. Feb. 19. Hundreds of tiny zoogl[oe]ae are now present. Feb. 23. Sharp line of demarcation in the U. Mar. 1. Like tube of grape sugar, but more cloudiness and more precipitate. Mar. 12. Quite cloudy, more so than in any of the preceding tubes and very decidedly more than in the tubes of milk sugar or of maltose; no pellicle; copious, pale yellow precipitate; this dextrin seems to be a particularly good food. Mar. 20. No change.	Perfectly clear	do	do	do	Do.
Do.	Mannitol (1 per cent Merck's c. p., in which the polariscope showed no contamination).	Feb. 15 (third day). Like the first except no zoogl[oe]ae visible. Feb. 23. Sharp line of demarcation in the U. Mar. 12. No pellicle; no visible precipitate; clouding slighter than in tube of grape sugar, i. e., like that in tubes of milk sugar and of maltose. Mar. 20. No change.	do	do	do	do	Do.
Do.	Glycerol (1 per cent Schering's twice distilled, sp. gr. 1.260, at 30.8°, Bé. strictly c. p.).	Feb. 15 (third day). Like tube containing the grape sugar, but no zoogl[oe]ae visible. Feb. 19. Still no zoogl[oe]ae, otherwise like tube of grape sugar. Mar. 12. Moderately cloudy, only a slight precipitate. Mar. 20. No change.	Feb. 19. Clear. Feb. 23. Very faintly clouded (like tube of maltose). Not yet certain what this faint cloudiness means. Mar. 1. As before, probably to be recorded as clear. Mar. 12. Perfectly clear.	do	do	do	Do.

Each of these tubes was inoculated February 12 with one loop from a beef broth culture made February 5. This culture was well clouded and becoming moderately turbid from the presence of numerous small zooglœae. It also contained a moderate amount of yellow precipitate. The tubes were very clear when inoculated, and perfectly sterile, the third steaming having taken place some weeks previous. The fluid in each tube was feebly alkaline to litmus when inoculated. The cultures were kept at living-room temperatures (20° to 23° C.). February 19 the cultures were first tested with litmus (the best neutral litmus paper procurable). The fluid in each was plainly alkaline (much more so than on the start). March 1 the fluids were again tested. All were alkaline to neutral litmus paper. March 12 the cultures were again tested. Each was plainly alkaline, although not strongly so. The blue color faded out when the paper dried. If any acid was formed it was masked by the alkali originally present in the tubes and by that produced during the growth of the organism.

The above results were obtained in 1897. In 1899 additional fermentation-tube experiments were instituted with the following results:

(1) One of the fluids used was a 1:2 nonpeptonized beef bouillon (stock 382) rendered neutral to phenolphthalein with sodium hydrate and deprived of its muscle sugar by growing *Bacillus coli* in it over night. It was then cleared by passing it through a Chamberland filter. The following substances were tested in this bouillon: Grape sugar, cane sugar, and galactose (3 tubes of each). Each tube contained 5 per cent of the sugar to be tested except those with the grape sugar which contained 2.8 per cent. The inoculations were made February 2 and the experiment was closed March 4. The tubes of grape sugar and cane sugar were all well clouded (in the bowl and outer two-thirds of the U) on the fifth day, with exception of one of the grape-sugar tubes which was then only very feebly clouded, but was well clouded 2 days later. In each case the closed end of the tube and the inner one-third of the U remained clear until the end of the experiment. The reaction to litmus was watched carefully. The fluid in the bowl of each of the tubes was plainly alkaline to litmus paper (wet or dried) on the ninth, fifteenth, and twenty-third days. On the thirtieth day in each tube, whether of grape sugar or cane sugar, the litmus reaction was distinctly different. The tests were made with two freshly prepared sensitive litmus papers, the one purplish red, the other pale lavender blue. The fluids now blued the purplish red paper slightly and at the same time reddened the bluish paper. The contrast in each case to inoculated check tubes of the plain bouillon (which were now intensely alkaline and blued both papers) was striking. The only conclusion I could come to was that a definite but small amount of acid had been formed slowly from the grape and the cane sugar. In comparison with plain bouillon these

sugar bouillons stimulated growth. No gas was formed. The growth in each was typical for *Ps. hyacinthi*.

In 2 of the 3 tubes of galactose *Ps. hyacinthi* refused to grow, and in the third clouding did not appear until after the seventh day. On the ninth day the fluid in the bowl and outer two-thirds of the U was feebly clouded. After a time there was an abundant yellow growth on the open end of the tube, but the closed end remained clear throughout the experiment. The fluid, as we have seen, was neutral to phenolphthalein (strongly alkaline to litmus) on the start. It was still strongly alkaline to litmus on the ninth day; on the fifteenth day it was moderately alkaline. On the twenty-third day it was neutral to litmus or nearly so, but so was an uninoculated tube. On the thirtieth day the fluid was distinctly acid, even to the purplish red paper. No gas was produced. The cloudy fluid was now pipetted from the open end of the bulb into a clean test tube and reduced by boiling to one-third its original volume. Moistened litmus paper was reddened in the vapors which first came off (CO_2?). Afterwards there was no reddening of the litmus paper in the steam and the concentrated fluid was more acid than before.

The fact that the organism failed to grow in two of the tubes and was retarded in the third was attributed to the effect of a soluble brown substance which appeared in the tubes as a result of the 3 steamings which followed the addition of the galactose.

(2) Absolute ethyl alcohol was also pipetted into 4 tubes of the same stock. Two of the tubes received 2½ per cent and two 5 per cent of this alcohol. Each tube was then inoculated with two 3 mm. loops from fluid cultures 13 days old (tubes 1 and 2, January 20, 1899). This experiment was suggested by the results obtained with Sharp and Dohme's litmus solution in milk. In one of the 5 per cent alcohols the organism failed to grow. In the other 3 tubes clouding occurred on the fifth to the seventh day; i. e., growth was retarded very decidedly. The tubes never became heavily clouded; growth ceased early and the closed end remained clear (30 days). The fluid was plainly alkaline to litmus at the beginning and on the ninth and the fifteenth days. On the latter date the appearance of the cultures was that of simple toleration of the alcohol rather than of any use of it for growth. The alkalinity in one of the 2½ per cent tubes on the fifteenth day was rather feeble; i. e., much less than in an uninoculated tube or than in inoculated tubes of the simple bouillon. On the twenty-third day the fluid had settled clear and was feebly acid to litmus. On the thirtieth day the fluid (in each tube) was clear and was distinctly acid to both the litmus papers. No gas had formed. The precipitate was distinctly yellow but scanty; i. e., there was only about one-twentieth to one-fiftieth as much as in the tubes of simple bouillon and about one one-hundredth as much as in the tubes of grape sugar and cane

sugar. The least growth was in the 5 per cent alcohol. Evidently the acid which was formed inhibited growth, although it did not immediately kill all of the organisms. This was determined by making 6 cultures from the 5 per cent alcohol on the twenty-third day (2 carrot, 2 potato, and 2 coconut cultures—1 loop for each). The organism grew in all these tubes, but its development was slow. It was not visible in any of them on the fourth day. The yellow growth appeared in 5 of these tubes on the sixth day and in the sixth tube a day or two later. A fact which shows the remarkably slow diffusion of the acid is that the fluid in the closed end of the tubes (2½ per cent alcohol) remained alkaline while that in the open end became acid. On the thirtieth day, in 1 tube, the fluid in the bowl was "distinctly acid to the blue paper and also to the pale red paper;" in the other it was "strongly acid to both red and blue papers." Nevertheless, when the contents of these 2 tubes was poured out into a clean test tube and thoroughly mixed it was no longer acid to either paper, but had become slightly alkaline; i. e., not enough acid was produced in the open end of the tube to neutralize the sodium hydrate in the 25 c. c. of fluid (25 c. c. of $\frac{N}{1}$ NaOH per liter). This fluid was then reduced one-half by boiling, but no acid vapors appeared in the steam.

(3) The experiments with glycerol and maltose were repeated to see whether the faint clouding which finally appeared in the closed end, in the experiments of 1897, should be attributed to facultative anaërobism or only to some accident. The stock used was a 1:2 slightly alkaline non-peptonized sugar-free beef bouillon (No. 450).[1]

To this was added 2 per cent of Schering's twice distilled c. p. glycerin in the one case and 2 per cent of Merck's c. p. maltose in the other. The experiments were carried through in duplicate. Having been on the shelf 15 days since the last sterilization, the tubes were resteamed for 20 minutes but no air bubbles appeared. Each tube was then inoculated with one 3 mm. loop from a cloudy broth culture 3 days old. The observations were continued 23 days.

Result.—The tubes of glycerin bouillon clouded in the bowl and outer three-fourths of the U on the second day, but remained entirely clear in the closed end during the whole time. The glycerin gave no increased clouding, i. e., not more than the simple bouillon. The line of demarcation in the U remained sharp. The fluid was slightly alkaline to litmus when inoculated and was neutral to feebly alkaline at the close of the experiment.

The maltose bouillon was feebly clouded in the bowl and outer three-fourths of the U on the second day. The line of demarcation in the U was sharp on the third day. On the seventh day the bowl and outer three-fourths of the U were uniformly and well clouded. This clouding was decidedly more than in the corresponding tubes of glycerin bouillon. The closed end and inner one-fourth of the U were still perfectly clear. On the twelfth day the line of demarcation in the U was less distinct and there was a faint haze in the lower part of the closed end (3 cm.). On the twenty-third day the faint haze had involved the whole of the closed end, but had not become any denser, i. e., the clouding in the closed end was not one one-hundredth part that in the open end. The fluid was feebly alkaline at the beginning of the experiment and was decidedly acid (to neutral litmus paper) at the close, i. e., the reaction was in marked contrast to that of the glycerin bouillon. Both tubes of the maltose bouillon behaved alike. They had been protected from jarring and inequalities of temperature, and steaming for 50 minutes at the close of the experiment did not cause the formation of any air bubble in the closed end. This very feeble clouding in the closed end after the second week would seem therefore to be due either to some contaminating substance in the maltose or else to that substance itself.

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[1] Freed from muscle sugar by *B. coli* and clarified with white of egg.

(4) The nitrate bouillon (stock 474) was also tested in fermentation tubes. Two tubes were inoculated from solid cultures 7 days old. Both clouded on the second day; both remained entirely clear in the closed end and inner one-fourth of the U until after the eighth day. On the fourteenth day both were feebly clouded in the whole of the closed end. No gas was formed and the fluid remained strongly alkaline.

On steaming these two tubes a bubble appeared in the closed end of each, and the feeble clouding was consequently attributed to growth stimulated by the presence of air absorbed from the open end.

The closed end of fermentation tubes filled with the following substances and inoculated with *Ps. campestris* remained entirely free from clouding: Potato broth; cabbage broth; cauliflower broth; peptone water with grape sugar, fruit sugar, cane sugar, milk sugar, galactose, maltose, dextrin, and glycerin. The open end clouded.

Dibasic calcium phosphate added in 5, 10, 20, and 30 milligram doses to test tubes holding 10, 15, and 20 c. c. of a peptone water containing grape sugar and glycerin, doubled the growth of *Ps. campestris.* Other species were not tried. This fluid was then tested in fermentation tubes. The calcium salt stimulated growth in the open end, but the closed end remained clear for three weeks. Afterwards there was clouding. This stock consisted of 200 c. c. of filtered Potomac water, 2 grams of Merck's c. p. anhydrous grape sugar, 4 c. c. of Schering's glycerin, and 2 grams of Witte's peptonum siccum; the whole dried out one-half by long standing and diluted with three times its bulk of distilled water before filling into the tubes and adding the phosphate.

Dibasic sodium phosphate used in the same stock also favored the growth of *Ps. campestris.*

From the above account it will be seen that in various ways the behavior of *Ps. hyacinthi* in fermentation tubes closely resembles that of *Ps. campestris.* *Ps. phaseoli* has not been tested so extensively but reacts in the same way, so far as tried, i. e., it produces no gas and is strongly aerobic. *Ps. stewarti* produces no gas and appears to be strictly aerobic, but is able to get along with a relatively small amount of air. A small amount of some non-volatile acid or acids appear to be produced by it from grape sugar, cane sugar, galactose, and mannitol, but not from glycerol.

GROWTH IN NITROGEN.

The tests were made in U tubes holding 250 c. c. and open at each end. Two very short cotton-plugged test tubes containing the freshly steamed culture medium were inoculated with *Ps. hyacinthi* and thrust, one above the other, into one arm of the U tube, which was then tightly closed with a soft rubber stopper and plunged, for greater security, into a beaker of glycerin. Into the other arm was thrust quickly a longer test tube filled with a mixture of pyrogallic acid, caustic potash, and water. This end of the tube was then plunged into a beaker of mercury and held down until the absorption of oxygen equalized the pressure and enabled it to remain down of its own weight—a period of some hours. The following experiments were tried in these tubes:

(1) The first experiment was with cylinders of freshly prepared coconut, a medium on which this organism was known to grow without retardation. Four tubes were inoculated. Two received each one loop of yellow slime from a solid culture 7 days old, which slime was rubbed carefully over the whole surface. Two received each two loops of fluid from the bottom of a potato (?) culture 7 days old, after shaking. One tube of each set was held as a check. The other 2 tubes were put into one arm of a U tube the other end of which received a tube holding 2 grams of pyrogallic acid and 25 c. c. of 13 per cent caustic potash water. The room temperature during the experiment ranged from 17° to 26° C. The oxygen was gradually absorbed and the tubes remained exposed to the nitrogen for 15 days.

Result.—In 48 hours from the time of inoculation the check tubes showed a good growth. On the eighth day the check cylinders were covered with an abundant, smooth, wet-shining, canary-yellow growth. In each tube there was at least 6 sq. cm. of this growth. During the same time, in the tubes exposed to the nitrogen, there was no visible growth.

On the fifteenth day the mercury seal was broken and the tubes were taken out and examined more critically. One tube showed no growth whatever and the other an extremely slight pale-yellow growth, best seen with a hand lens, and aggregating not over one-fourth of 1 sq. mm., i. e., not more than might have grown around one of the coarser fragments of the inserted slime before all of the oxygen was absorbed. At this time the contrast with the checks was very striking. The tiny bacterial mass referred to contained no chains, no spores, and no involution forms. It consisted of slender rods, single or in pairs and very short, as if not now dividing. Exposure of these rods for 10 minutes to a temperature of 74° C. falling to 60° C. killed all of them.

The unexpected feature of this experiment was that after removal to the air growth did not appear in these tubes as soon as it did in the check tubes; in other words, the sojourn in the nitrogen seemed to have exerted an injurious influence. One of the tubes (that inoculated from the solid culture) showed a slight growth at the end of the third day, the other one not until the fifth day. Five days after removal from the nitrogen the bacteria in one tube had made about as much growth as the check tubes made in 48 to 60 hours. In the other tube they had made a thin pale yellow growth covering not more than 1 sq. cm.—i. e., not more than one-tenth as much growth as the check tube made in the same time. In the course of another 3 or 4 days the bacteria in both tubes made an abundant bright yellow growth.

Ps. stewarti tested at the same time behaved in the same way. At the end of 15 days, when the seal was broken, there was no yellow precipitate or visible slime, colored or colorless, in either tube. The two check tubes showed a distinct growth in 48 hours, and continued to grow in a typical way. On the contrary, there was no visible growth in either tube on the fourth day after removal from the nitrogen. On the fifth day in the tube which was inoculated from a solid culture there was a slight yellow growth over a few square millimeters. In the other tube no growth was visible until the eighth day after the removal, and then it was scanty. This cylinder stood in one-half c.c. water and was still moist. Two days later there was a good growth on both cylinders.

(2) The stock in the second experiment with *Ps. hyacinthi* consisted of 6 tubes of white turnip. Each of 3 was inoculated with one loop of a very cloudy beef-broth culture 6 days old. Each of the other 3 was inoculated with one loop of very cloudy fluid from the bottom of a young bright yellow and very vigorous culture on coconut after prolonged shaking.. Two of the tubes were held as checks. The other 4 were put into 2 U tubes in the way already described. In each case a test tube (capacity 25 c. c.) packed nearly full of pyrogallic acid was then filled with 6½ per cent caustic potash water and immediately thrust into the other arm of the tube, which was then plunged into the mercury. By the end of 24 hours, and probably sooner, the absorption of the oxygen was complete—i. e., there was no farther rise of the mercury or change in the color of the pyrogallic acid.

Result.—In one of the check tubes growth was plainly visible on the third day, in the other not. On the sixth day in one check tube there was an abundant smooth, wet-shining growth over the whole cylinder out of the water; the fluid was also heavily clouded, and there was considerable pale yellow precipitate. In the other check tube growth was not so abundant, but about 3½ sq. cm. of the slant surface was covered with a smooth, wet-shining, pale yellow growth.

The tubes were removed from the nitrogen on the fifteenth day. In none of the 4 had there been any growth whatever, although there was an abundance of moisture in each. Moreover, in none of them did any growth subsequently appear (17 days).

The pyrogallic acid used in this instance was a fresh supply and had a peculiar penetrating smell. Whether the failure of these cultures to grow after removal to the air is to be ascribed to the nitrogen or to some substance emanating from the pyrogallic acid must be left an open question.

Ps. campestris and *Ps. stewarti* were tested at the same time with identical results. The check tubes grew promptly. The others (2 of *Ps. campestris* and 4 of *Ps. stewarti*) made no growth whatever, either while in the nitrogen (15 days) or after being taken out (17 days). The temperature during this experiment ranged from 20° to 25° C.

(3) The third experiment did not fully accomplish what was intended, but is perhaps just as instructive. Each U tube received a tube containing 10 grams of an old stock of pyrogallic acid, not previously used, and 20 c. c. of 5 per cent caustic potash water. It browned slowly, and at the end of 48 hours a considerable part of the oxygen remained unabsorbed (perhaps one-third), and meanwhile the bacteria had begun to grow. The cultures were on coconut. Each tube was inoculated with two 3 mm. loops of *Ps. hyacinthi* from a cloudy beef-broth culture 5 days old.

Result.—The check tube grew promptly. During the first 46 hours the bacteria in the two tubes in the nitrogen (+ some oxygen) made about one-half as much growth as in the check tube. The column of mercury was now 40 mm. high. There was some additional growth in these tubes on the third day, but it was paler yellow than in the check tube. At the beginning of the fifth day the mercury stood at 58 mm. and the oxygen was probably all absorbed. From this time on there was no increase in growth. On the fifteenth day the seal was broken and the tubes removed for a more careful examination. The pale yellow growth in each tube was not more than one-twentieth as much as in the check tube.

The results were much the same in another U tube. At the end of the second day the mercury stood at 35 mm. At the beginning of the fifth day it had reached 59 mm. During this very gradual absorption of the oxygen there was some growth, but it was less than in the check tube (not over one-fifth as much), and it ceased after this date. The color of the slime in the check tube at this time was canary yellow.

The color of the slime in the tubes from the nitrogen was paler, i. e., between primrose and Naples yellow. This U tube was also opened on the fifteenth day, at which time the growth was still pale yellow and not over one-thirtieth as abundant as in the check tube.

In 8 days from the time these 4 tubes were removed from the nitrogen there was an abundant, smooth, wet-shining, bright yellow growth in each tube. This new growth began to be visible at the end of the second day. That a considerable portion of the germs were injured by exposure in the U tube was, however, shown by the fact that scrapings taken from the rather dry bacterial layer in each one of these tubes when they were first opened and put into as many tubes of beef broth failed to cloud them in 8 days.

Ps. campestris and *Ps. stewarti* were tested at the same time. *Ps. campestris* was grown on cylinders of flat white turnip in distilled water and *Ps. stewarti* on similar cylinders of sugar beet, i. e., each one on a medium specially adapted to its growth. In the check tubes growth was prompt and abundant.

In the U tube containing *Ps. campestris* the mercury had risen only 30 mm. in 46 hours, and there was nearly or quite as much growth in these tubes as in the check. On the beginning of the fourth day the mercury stood at 50 mm., and the growth was comparatively feeble, i. e., not one-twentieth as much as in the check. On the fifteenth day when the seal was broken the slime had dried away and there was no apparent growth in either tube. Eight days later each cylinder was covered with a copious pale yellow, smooth, wet-shining slime which also filled the fluid. This increased growth began to be visible the second day. A second U tube gave identical results.

In the U tube containing *Ps. stewarti* the mercury had risen only 15 mm. in 46 hours and there was about as much growth as in the check. At the beginning of the fourth day the mercury stood at 50 mm., and the growth was now not one-fifth as much as in the check tube. At the beginning of the fifth day the mercury stood at 55 mm., i. e., nearly all of the oxygen was absorbed and the growth was not one-tenth as much as in the check tube. At this time the color of the growth in the check tube was between buff yellow and deep chrome. that in the tubes in the nitrogen was "pale yellow." On the fifteenth day when the seal was broken there was not in either of these tubes over one-thirtieth as much growth as in the check, and it was paler yellow. In the fluid in the bottom of the check there was also a copious buff-yellow precipitate, but there was none in either of the tubes which had been in the nitrogen. Here, again, something seems to have done injury to the organisms, for after breaking the seal and exposing them to the air there was little increase in growth (8 days). The check was deep buff yellow. In the tubes which had been in the nitrogen there was no buff yellow, but only a thin whitish growth. These 3 tubes were each inoculated in the same way, i. e., with 2–3 mm. loops from a beef-broth culture 5 days old.

GROWTH IN VACUO.

(1) The first test was in a partial vacuum with the remnant of the oxygen absorbed. Under the bell jar with the cultures was a beaker containing 5 grams of pyrogallic acid. In this beaker was a U tube, the short arm open, the long arm closed, and containing 30 c. c. of 13 per cent caustic potash water, with a small bubble of air at the top. The size of this bubble was so regulated that its expansion would begin to force over the potash water into the pyrogallic acid when four-fifths of the air was exhausted. The exhaustion was continued until the mercury in the cistern barometer stood at 2¼ inches. The stopcock was then turned and the apparatus separated from the pump, well sealed, and put away in the dark. The temperature during the experiment was 20° to 26° C.

Eight test-tube cultures of *Ps. hyacinthi* were subjected to this experiment. Four were on coconut (stock 395), each being inoculated with one loop of yellow slime from tube 27, February 2. Four were on potato (stock 385), each being inoculated with two loops of fluid from the bottom of tube 29, February 2, after long shaking. Two tubes of each set were placed under the bell jar and the other 4 tubes were held as checks. The experiment was begun on February 9 and the seal of the jar was broken February 18, at which time the vacuum continued as perfect as when first made.

Result.—The 4 check tubes each showed a distinct yellow growth at the end of 48 hours, and this growth continued in a typical manner. The 4 tubes in the vacuum showed no growth whatever at the end of the ninth day, when the vacuum was broken. Twenty-four hours later there was no visible growth in any of these tubes. At the end of 48 hours the 2 potato cultures showed no growth; the coconut cultures showed a slight yellow growth on the inoculated face. At the end of the third day the coconut cultures showed two or three times as much growth as at the end of 48 hours, but the growth was still thin and did not cover all of the cylinder, i. e., was not more abundant than the growth in the check tubes at the end of the third day. One of the potato cultures now showed a feeble yellow growth (less than the check tube showed at the end of the second day), and there was still no visible growth or graying of the substratum in the other tube. Six days after removal from the vacuum there was a moderately abundant bright canary yellow slime covering all that part of the coconut cylinders which projected out of the water. One of the potato cultures now contained about as much growth as the check tube, while the other also showed some growth (4 sq. cm.). In other words, there was no growth in the oxygen-free vacuum; and 9 days' exposure to it while not killing all of the organisms probably killed many of them, since subsequent growth in the air was distinctly retarded.

Cultures of *Ps. stewarti* and of *Bacillus amylovorus* were also exposed to this vacuum. *Bacillus amylovorus* was inoculated on ordinary slant beef extract peptone agar. On this substratum, which probably contained a little muscle sugar, it made a slight but distinct growth. The check tube developed promptly, and made a good white growth the whole length of the streak. The growth in vacuo was about one-tenth to one-fifteenth as much as in the air. The 4 check tubes of *Ps. stewarti* (2 coconut, 2 potato) developed a distinct buff yellow growth within 48 hours. The 4 tubes in vacuo made no growth whatever during the 9 days' exposure, and after removal to the air growth in each one was even more distinctly retarded than in case of *Ps. hyacinthi*.

(2) The second test was made in the same manner as the first, except that the vacuum was not so complete and the remnant of oxygen was not removed. The experiment was begun March 8, and the seal was broken March 20. The mercury in the cistern barometer was down to 3 inches when the jar was sealed, and the vacuum kept quite well. The temperature during the experiment was 16° to 25° C. (mostly 20° to 22° C.).

Four organisms were tried in this jar, *Ps. hyacinthi*, *Ps. campestris*, *Ps. stewarti*, and *Bacillus carotovorus*. The media used were carrot (stock 402), alkaline beef broth (stock 382), coconut (stock 412), and potato (stock 406). All of the check tubes but one made a "feeble" to "good" growth within 48 hours, and all showed a "good growth" at the end of the third day except one tube of *Ps. hyacinthi* on potato, which lagged and was doubtful, but which 2 days later showed the typical yellow growth over about 4 sq. cm. The tubes in the vacuum were distinctly different. On the fifth day *Ps. hyacinthi* showed some growth on coconut and potato, but it was not as yellow as in the air. The same was true of *Ps. campestris* and *Ps. stewarti*. Each showed some growth, and neither was as yellow as in the checks. On the eighth day the mercury stood at 3¾ inches, and none of the potato cylinders were grayed. The condition on the twelfth day (March 20), when the seal was broken, and on subsequent days, was as follows:

Ps. hyacinthi:

(a) *Carrot.*—March 20, no visible growth (there was no check upon this tube); March 23, no growth; March 31, no growth; April 5, a smooth, wet-shining, translucent growth now covers the whole exposed surface of the carrot, and the precipitate is yellow; April 17, slime and fluid distinctly acid.

(b) *Beef broth.*—March 20, fluid clear, precipitate very slight (2 mm. broad), colorless; no rim, no pellicle, no zoogleæ; March 23, feebly clouded; March 31, well clouded.

(c) *Coconut.*—March 20, a very thin pale-yellowish growth, not one one-hundredth as much growth as in the check tube; the difference in color was not due to unlike volumes; bulk for bulk on white paper the slime from the check tube was yellower than that from the tube exposed to the vacuum. March 23, a thin growth covers 5 to 6 sq. cm.; it is yellow, but rather pale for this substratum. March 31, a bright-yellow growth now covers most of the aerial portions of the cylinder.

(d) *Potato.*—March 20, a distinct but feeble pale-yellow growth; about one-tenth as much growth as in the check tube; the potato has not grayed; bulk for bulk on white paper the slime from the check tube is yellower; compared as a whole the culture in the check tube was a canary yellow; that from the vacuum was primrose yellow. March 23, a thin growth covers 2 to 3 sq. cm.; it is yellow, but seems unusually pale.

Ps. campestris:

(a) *Carrot.*—March 20, a very feeble growth; no check tube. March 23, a feeble, wet growth which does not mask the color of the carrot. March 31, a feeble growth; substratum not hidden. April 17, slime and fluid distinctly acid.

(b) *Beef broth.*—March 20, clear; a very slight white precipitate closely resembling that of *Ps. hyacinthi;* not over one-twentieth as much precipitate as in the corresponding tube of *Ps. stewarti.* March 23, feebly clouded; March 31, well clouded.

(c) *Coconut.*—March 20, a thin, pale-yellow growth over the whole aerial part of the cylinder; about one-fiftieth as much growth as in the check tube, and paler yellow; the difference in color was also apparent when equal volumes of the slime were placed side by side on white paper. March 23, 8 to 9 sq. cm. of rather pale-yellow slime. March 31, a distinctly yellow growth over the whole exposed surface.

(d) *Potato.*—March 20, the fluid is moderately cloudy and a thin, very pale-yellow growth covers the whole aerial part of the potato; there is no precipitate, no graying of the potato, no thickening of the fluid or color in it; the check culture is much yellower and contains fully 100 times as much growth; the check tube is wax yellow; the other culture is as pale as primrose yellow; side by side on white paper in equal quantities the slime of the check tube was yellower. March 23, the entire aerial part of the potato is covered with a yellow slime which also begins to fill the water; it is still rather pale but begins to recover its color and vigor. March 31, a copious typical growth.

Ps. stewarti:

(a) *Carrot.*—March 20, only the slightest trace of growth; no check tube. March 23, a slight growth, scarcely visible. March 31, fluid well clouded; out of the water there is a thin slime which does not hide the carrot. April 17, slime and fluid distinctly alkaline.

(b) *Beef broth.*—March 20, fluid very feebly clouded; a pale-yellow precipitate, 6 mm. in breadth, i. e., more than in the corresponding tubes of *Ps. hyacinthi* and *Ps. campestris;* check tube twice as cloudy and with double the precipitate, which is yellower; this organism seems to be able to get along with less oxygen than *Ps. hyacinthi* or *Ps. campestris.* March 23, fluid feebly clouded; cloudier than when taken out. March 31, well clouded.

(c) *Coconut.*—March 20, a very thin, very pale-yellow growth; the check tube contains several times as much growth and it is yellower; the one is buff yellow, the other is cream (Ridgway); removed from the tube and examined bulk for bulk and side by side on white paper, the slime from the exposed tube was also distinctly paler. March 23, a very thin, buff-yellow growth covers 4 to 5 sq. cm; it is paler than usual. March 31, there is now a thin, buff-yellow layer over the whole exposed surface.

(d) *Potato.*—March 20, a pale buff-yellow growth about one-third to one-half as abundant as in the check tube; potato not grayed, color only a little paler than that in check tube. March 23, a very thin, pale buff-yellow growth covers 4 to 5 sq. cm; the slime is very pale yellow for the amount of growth; the potato in the air begins to gray. March 31, growth feeble.

Growth in Hydrogen.

Two tests were made in hydrogen. The gas was prepared by the action of zinc on c. p. sulphuric acid dissolved in distilled water (acid 1 part, water 9 parts). It was produced in quantity in a Kipp generator and was freed from impurities by passing it through strong solutions of argentic nitrate, potassium permanganate, and sodium hydrate. It was finally allowed to bubble through a jar of distilled water and then passed into the culture chamber. This zinc was certified to be free from arsenic and subsequent tests did not reveal any of this substance. To facilitate the removal of air, the gaseous contents of the well-luted bell jar was pumped out before allowing the hydrogen to enter. The jar was then repeatedly pumped out and refilled with the hydrogen, so that only a trace of oxygen could have remained. During the preliminary trial exhaustions, leaks were of course discovered in various places and were waxed or screwed tight. At the beginning of each experiment everything was gas tight and remained so until its close (16 days). The exposures were in a large Novy jar. At the close of each experiment the tightness of the seal was demonstrated by the fact that when the 4 clamp screws were loosened hydrogen passed out through the broad vaselined rubber joint (with a slight sound) in hundreds of tiny branching whitish rivulets and then air began to pass into the jar in the same curious way.

(1) The first experiment was begun June 14 and closed June 30. The temperature during this period was the ordinary room temperature of Washington (usually 25° to 30° C. in June). The inoculations were all into test tubes, using in case of each tube and of each organism one 2-mm. loop of cloudy beef broth 3 days old. The culture media tested were potato (stock 519), +15 beef broth (stock 473a), and +15 nutrient slant agar (stock 516), i. e., media well adapted to these organisms. Various bacteria were tested. The observations on opening the jar June 30 (sixteenth day) and on subsequent days are given below:

Ps. hyacinthi:

(a) *Potato.*—June 30, no growth. July 2, no growth; plenty of water in the tube. July 5 (end of fifth day), doubtful; there seems to be feeble clouding and a slight growth on the potato out of the water. July 9, distinct feeble, pale-yellow growth; potato grayed; fluid feebly browned. July 16, a thin, yellow, typical growth covers a portion only of the exposed potato; there is also a small amount of yellow precipitate; fluid abundant; a marked retardation of growth.

(b) *Beef broth.*—June 30, clear; no growth. July 2, clear. July 5, no growth. July 9, not cloudy; July 16, clear; no growth.

(c) *Agar.*—June 30, no growth. July 2, no growth. July 5, no growth. July 9, no growth. July 16, no growth; the agar is still quite moist, i. e., it has dried out only a little; this tube and the two preceding were inoculated from the same culture, tube 4, June 11 (stock 473a), which was well clouded.

Ps. campestris:

(a) *Potato.*—June 30, no growth. July 2, no growth. July 5, no growth. July 9, no growth. July 16, no growth; plenty of water in the bottom of the tube and the aerial part of the potato moist; this tube and the two following were inoculated June 14 with a large 2-mm. loop from tube 13, June 11 (a beef-broth culture inoculated with a 2-mm. loop of yellow slime from a potato culture 36 days old); tube 13, June 11, clouded in 24 hours, and was well clouded in 48 hours.

(b) *Beef broth.*—June 30, clear; no trace of growth. July 2, clear. July 5, lost by accident.

(c) *Agar.*—June 30, no growth. July 2, no growth. July 5, no growth. July 16, no growth; failure is not to be accounted for by any drying out of the agar.

Ps. stewarti:

(a) *Potato.*—June 30, no growth. July 2, no growth; plenty of water in the tube. July 5, no visible growth. July 9, potato grayed, a very feeble buff-yellow growth, not over one-fiftieth as much as in the corresponding tube of *Ps. hyacinthi.* July 17, a feeble buff-yellow growth in the air; potato quite gray; fluid feebly browned; very little precipitate; a marked retardation of growth; this culture and the two following were inoculated from tube 22, June 11 (stock 473a), a well-clouded culture.

(b) *Beef broth.*—June 30, clear; no growth, or a very slight one which has settled; the nature of the slight precipitate is doubtful; it was not examined microscopically; it is possible that a trace of oxygen was left in the medium, and this organism seems to require less O. for its growth than *Ps. hyacinthi* or *Ps. campestris.* July 2, fluid feebly clouded; no rim or pellicle, but many small zooglœæ. July 5, well clouded. July 9, well clouded; no pellicle or rim. July 16, moderately cloudy, no rim or pellicle, but numerous small zooglœæ and a moderate amount of yellow precipitate; no decided retardation of growth in the air.

(c) *Agar.*—June 30, a distinct but very feeble growth; it is visible to the naked eye in the V and on the slant, if the tube is held up to the light, but it is best seen with a Zeiss × 6 aplanat; under this magnification there appear to be 300 or 400 tiny whitish colonies on the slant surface, and in the fluid a feeble clouding and some tiny zooglœæ; no yellow color is visible. July 2, a streak composed of several hundred small white colonies barely visible to the naked eye. July 5, a pale yellow growth now covers about 1 sq. cm. in the lower part of the streak. July 9, some increase of the yellow growth, but not over one-third of the slant covered; farther up there are more than 100 minute colonies, which can be seen only with a lens. July 16, growth on the lower part of the slant has doubled and is yellower than it was (buff-yellow); the tiny colonies on the middle and upper part of the slant have not increased any in size; they are dead; the agar has dried out but little.

B. pyocyaneus-pericarditidis:

(a) *Potato.*—June 30, no visible growth. July 2, an abundant growth, whitish with a tinge of yellow; no fluorescence. July 5, potato grayed throughout. July 9, a thin dirty white (or brownish white) growth; no fluorescence; growth in the air not retarded by the hydrogen.

B. amylovorus:

(a) *Potato.*—June 30, no growth. July 2, fluid well clouded and a distinct white growth on the potato out of the water. July 5, fluid heavily clouded; potato feebly grayed; growth in the air not retarded.

B. coli:

(a) *Potato.*—June 30, fluid well clouded, doubtful as to growth out of the water; if any, it is slight and of the same color as the potato; potato not grayed. July 2, scanty wet-shining white growth on the potato out of the water; organism will grow in hydrogen.

(2) The second test was begun June 16 and closed July 1. Four media were used, viz, +15 peptonized beef broth (stock 473a), peptone water with addition of grape sugar and methylene blue (stock 489), peptone water with sodium chloride and rosolic acid (stock 493), and Uschinsky's solution (stock 496). Various organisms were tested. Each tube received an equal quantity of the culture fluid, i. e., one 2 mm. loop of cloudy broth from cultures five days old. The media used had already been tested and the various organisms were known to grow well in it. The inoculations were all made from media in which the various organisms grew well, viz, peptonized beef broth neutral to phenolphthalein (stock 515e). The general management of the experiment in other particulars was the same as in the preceding.

The seal was broken, as before, on the sixteenth day (July 1), and the results were as follows:

Ps. hyacinthi:

(a) *Beef broth.*—July 1, no growth. July 2, clear. July 5, clear. July 9, clear. July 16, feebly clouded; good rolling clouds on shaking; a great retardation of growth.

(b) *Grape sugar peptone water with methylene blue.*—July 1, no growth; on removal the fluid was nearly colorless, but the surface layer in contact with the air immediately became greenish blue and in a few minutes the whole fluid was oxidized to this color; this result also shows that the jar remained free from oxygen. July 2, clear, July 5, clear; July 9, clear; no visible growth. July 16, well clouded; color wholly reduced, except in a thin layer at the top next to the air; this growth and reduction of color began about July 12, on shaking, the color comes back, but is again reduced on standing for a few minutes; marked retardation of growth.

(c) *Salted peptone water with rosolic acid.*—July 1, no growth; color of the fluid the same as in the uninoculated tubes. July 2, clear. July 5, no growth. July 9, clear. July 16, no growth visible; no change of color.

Ps. campestris:

(a) *Beef broth.*—Lost by breaking.

(b) *Grape sugar peptone water with methylene blue.*—July 1, no growth; fluid nearly colorless when taken from the jar; on contact with the air it began to color at once and in a few minutes was greenish blue. July 2, clear. July 5, clear. July 9, clear. July 16, fluid greenish blue; no growth.

(c) *Salted peptone water with rosolic acid.*—July 1, no growth; the fluid is the same color as when inoculated. July 2, clear. July 5, no growth. July 9, clear. July 16, clear; no change in color.

Ps. stewarti:

(a) *Beef broth.*—July 1, no growth. July 2 (temperature 28° C.), very feebly clouded. July 5, moderate clouding, most in the upper 6 mm. where there are numerous small zoogloeæ which stream down on gentle shaking; much increase in growth since July 2, but no rim or pellicle; July 9, moderately cloudy; no rim, but a delicate pseudo-pellicle of separate zoogloeæ. July 16, feebly clouded.; a moderate amount of yellow precipitate and a thin fragile yellow iridescent pellicle, which breaks up on slight shaking into a great many roundish zoogloeæ; growth in the air not distinctly retarded.

(b) *Grape sugar peptone water with methylene blue.*—July 1, clear. There seems to have been a little growth—i. e., there are a few tiny floating flecks of uncertain nature, there is a small amount of colorless precipitate which is wanting in the corresponding tubes of *Ps. hyacinthi* and *Ps. campestris*, and the reduced fluid oxidizes back on contact with the air to a color which is bluer than that in the tubes already mentioned, and which resembles that in a tube of Jones's carrot rot organism (*Bacillus carotovorus*), where there has certainly been some growth. July 2, feebly clouded; rolling clouds on shaking. July 5, feebly clouded. July 9, clear or very nearly so; no rim or pellicle. July 16, clear; no reduction; fluid a pure blue; a feeble growth after removal to the air, but no marked retardation.

(c) *Salted peptone water with rosolic acid.*—July 1, no growth; fluid the same color as when inoculated. July 2, clear. July 5, there seems to be a slight deepening of the color, the clouding is not distinct. July 9, not much change. July 16, as on the 9th; fluid slightly pinker than in the corresponding tubes of *Ps. hyacinthi* and *Ps. campestris*; not cloudy.

(d) *Uschinsky's solution.*—July 1, no growth. July 2, clear. July 5, clear. July 9, clear. July 16, clear; no growth.

B. pyocyaneus pericarditidis:

(a) *Beef broth.*—July 1, a slight growth, which has not increased any of late; the fluid is clear and there is no rim, but there is a small amount of precipitate (10 mm. wide), and a bacterial film invisible to the naked eye, but distinct under the lens, and covering about one-sixth of the surface. July 2, moderately cloudy; fluid green fluorescent in the upper one-fourth and bearing a thick white pellicle. July 5, very heavy clouding and a marked increase of the fluorescence; an abundant white precipitate, a thin white rim, and a white pellicle which settles easily on jarring. July 9, very cloudy, but fluorescence not pronounced. July 16, fluid well clouded; very ropy; only slightly fluorescent, feebly browned; precipitate 4 mm. deep; no retardation of growth after removal to the air.

(b) *Grape sugar peptone water with methylene blue.*—July 1, no growth or only the merest trace; fluid nearly colorless; it becomes greenish in a few minutes on exposure to the air. July 2, fluid clouded, with a thin pellicle; color half reduced. July 5, fluid well clouded and uniformly blue, if any reduction of color it is uniform; some of the pellicle has fallen; under the fluid there is a thin white rim 1½ mm. wide. July 9, cloudy blue with a thin white rim and pellicle. July 16, the pellicle has fallen; the fluid is pure blue; there is no distinct fluorescence or reduction of color.

(c) *Salted peptone water with rosolic acid.*—July 1, a trace of growth; not cloudy, but with a slight precipitate and a membranaceous pellicle visible only under a lens; no rim. July 2, fluid clouded, pellicle more distinct; the color has turned toward pink. July 5, moderately cloudy; no pellicle, that of July 2 lies on the bottom unbroken; there is a thin rim under the surface of the fluid; the latter is now bright pink; it was originally yellowish rosaceous and the uninoculated tubes are still that color. July 9, much as on 5th. July 16, fluid moderately cloudy, color bright pink red.

B. amylovorus:

(a) *Beef broth.*—July 1, clear; no present growth; there is a slight precipitate, chemical (?); it is much less than in case of *B. coli*. July 2, clear. July 5, clear. July 9, clear. July 16, clear; no growth, unless possibly when first placed in the hydrogen.

(b) *Grape sugar peptone water with methylene blue.*—July 1, no growth; fluid nearly colorless when removed, but soon changing to a greenish blue, as in case of *Ps. hyacinthi* and *Ps. campestris*. July 2, clear. July 5, clear. July 9, clear. July 16, no reduction; no clouding; fluid "pure blue."

(c) *Salted peptone water with rosolic acid.*—July 1, no rim, pellicle, or clouding. A slight rosy precipitate (2 mm. wide), which is possibly chemical; the color of the fluid is the same as when inoculated. July 2, clear. July 5, no growth; no change in color. July 9, as on the 5th. July 16, as on the 5th.

B. coli:

(a) *Beef broth.*—July 1, some growth; no rim or pellicle; only the merest trace of clouding and no rolling clouds on shaking, but a white precipitate 10 mm. broad. July 2, well clouded; a thin white rim, and a gathering of zoogloeæ into the upper layers which are cloudiest. July 5, heavily clouded, more so than on the 2d; a thin white pellicle and a white rim 3 mm. wide. July 9, as on the 5th; the pellicle settles on very gentle shaking.

(b) *Grape sugar peptone water with methylene blue.*—July 1, a slight growth; fluid feebly clouded; no rim, but some slight fragments of pellicle and a precipitate 4 mm. wide; fluid nearly colorless; on exposure to the air the fluid becomes *bluish,* i. e., like the carrot-rot culture; the uninoculated tubes are greenish. July 2, heavily clouded; there has been no reduction of the color; it is now a pure bright blue (brighter than yesterday). July 5, well clouded; no rim or pellicle; fluid (by transmitted light) a uniform bright blue. July 9, as on the 5th. July 16, fluid pure blue, no reduction of color; moderately cloudy, no rim, no pellicle; a scanty bacterial precipitate which is *blue.*

(c) *Salted peptone water with rosolic acid.*—July 1, a rosy precipitate 3 mm. wide; no clouding, no rim, no pellicle. July 2, moderately cloudy; fluid is changing to pink. July 5, well clouded; no rim or pellicle; fluid deep pink; at least twice as much color as in the corresponding tube of *B. pyocyaneus pericarditidis.* July 9, as on 5th. July 16, feebly clouded, slight precipitate; no rim or pellicle; fluid deeper red than that in the corresponding tube of *B. pyocyaneus-pericarditidis.*

Growth in Carbon Dioxide.

The carbon dioxide was prepared in quantity in a Kipp generator from boiled marble chips and c. p. hydrochloric acid diluted with distilled water (1 part acid, 9 parts water). The gas was allowed to flow until all air was displaced from the apparatus. It was washed in 1 per cent caustic potash water and then in distilled water. The tubes were exposed in a deep specimen jar with a flat brass top provided with inflow and outflow tubes having very perfect stopcocks. When all was ready a waxed rubber gasket was laid on the top of the jar and the solid brass top was clamped down securely. The jar was first exhausted of air until the mercury stood at 3 inches. It was then filled with the CO_2 five times, and as many times pumped out. After the sixth filling the stopcock was turned off and everything sealed securely. Preliminary exhaustion tests had shown only a slight leakage, i. e., in 24 hours the mercury in the cistern barometer rose only from 2½ to 3½ inches.

The following media were tested: Tubes of beef broth neutral to phenolphthalein (stock 382); tubes of potato (stock 405); tubes of coconut (stock 412); slant beef-extract peptone agar neutral to litmus. Each tube was inoculated copiously and in the same way, i. e., with large loops from well-clouded beef broth cultures 13 days old. Two or more tubes of each medium were inoculated and one of each medium was held as a check. The exposure was begun March 10 and the tubes were removed to the air after 10 days, i. e., on March 20. On taking off the brass cover, lighted matches were repeatedly plunged into the jar and as often extinguished. They went out instantly they were depressed below the level of the top of the jar. The checks behaved well. On the sixth day in each one there was a well-developed typical growth of the particular organism used.

The results obtained in the CO_2 and on continuing the cultures in the air are stated below. The temperature during the experiment did not vary much from 22° C.

Ps. hyacinthi:

(a) *Beef broth.*—March 20, no growth. March 21, clear. March 23, no growth. March 27, moderately cloudy with rolling clouds on shaking; a slight precipitate. March 31, well clouded; growth retarded by the CO_2.

(b) *Potato.*—March 20, no growth. March 21, no growth. March 23, no growth. March 27, a typical growth, wet and distinctly yellow on the lower one-half of the exposed part of the potato; that part out of the water is graying. March 31, the yellow slime now covers all of the potato out of the water.

(c) *Potato.*—March 20, no growth. March 21, no growth. March 23, no growth. March 27, same appearance as in the preceding; growth retarded by the CO_2. March 31, like the preceding

(d) *Coconut.*—March 20, no growth. March 21, a thin yellow growth now covers about 1 sq. cm.; growth in the air not retarded by the exposure. March 23, a thin distinct yellow growth now covers 6 to 7 sq. cm. March 27, 9 sq. cm. of bright yellow growth.

(e) *Coconut.*—March 20, no growth. March 21, a thin, yellow growth now covers about 3 sq. cm. March 23, like the preceding. March 27, like the preceding.

Ps. campestris:

(a) *Beef broth.*—March 20, no growth. March 21, clear. March 23, no growth. March 27, no growth. March 31, no growth; fluid still alkaline; it was now reinoculated with a small amount of yellow slime from potato culture *b*, and on April 5 was well clouded with a yellow rim and numerous zoogleæ; exposure to CO_2 appears to have destroyed the organism.

(b) *Potato.*—March 20, no growth. March 21, no visible growth. March 23, a feeble pale-yellow growth now covers part of the potato; growth retarded by the CO_2. March 27, a copious wet-looking, distinctly yellow slime on the exposed parts of the potato and in the fluid.

(c) *Potato.*—March 20, no growth. March 21, no visible growth. March 23, resembles the preceding—less growth but more color; growth retarded. March 27, like the preceding.

(d) *Coconut.*—March 20, no growth. March 21, no visible growth. March 23, a thin yellow growth now covers 5 sq. cm. March 27, a yellow growth now covers nearly all the cylinder out of the water; no distinct retardation.

(e) *Coconut.*—March 20, no growth. March 21, no visible growth. March 23, a thin yellow growth covers 3 sq. cm. March 27, like the preceding.

Ps. stewarti:

(a) *Beef broth.*—March 20, no growth. March 21, clear. March 23, no growth. March 27, no growth. March 31, no growth; reinoculated with a small amount of slime from the coconut culture *d*. On April 5 the culture was well clouded and had a good rim; exposure to the CO_2 appears to have destroyed the organism.

(b) *Potato.*—March 20, no growth. March 21, no visible growth. March 23, a feeble, patchy, buff-yellow growth now covers 6 sq. cm. March 27, a typical buff-yellow growth; marked graying of the potato in the air.

(c) *Potato.*—March 20, no growth. March 21, no visible growth. March 23, a feeble, patchy, buff-yellow growth covers 3 sq. cm.; neither potato has grayed. March 27, like the preceding.

(d) *Coconut.*—March 20, no growth. March 21, extremely thin (barely visible) buff-yellow growth over 3 sq. cm. March 23, a thin, pale, buff-yellow growth now covers about 5 sq. cm. March 27, a rather scant buff-yellow growth over 8 sq. cm. No retardation.

B. amylovorus:

(a) *Agar.*—March 20, no growth. March 21, a distinct growth on the lower end of the slant. March 23, the white growth slowly increases.

This closes my studies of the aerobism of *Ps. hyacinthi* and related species. All the various experiments lead to substantially the same conclusions: (1) *Ps. hyacinthi* and the other yellow species of Pseudomonas are more strictly aerobic than most species of bacteria; (2) while somewhat variable among themselves none of these yellow-plant parasites will survive exclusion of oxygen for more than a very few weeks; (3) nitrogen, hydrogen, and carbon dioxide seem to be only negatively harmful; (4) the organisms were more tolerant of these gases on some media than in others. They were especially susceptible in beef broth, in peptone water, and on agar.

RELATIVE NUTRIENT VALUE OF CARBON COMPOUNDS.

Bouillon and Peptone Water with Various Sugars, etc.

The few results obtained may be summed up as follows:

(1) A feeble clouding was obtained with *Ps. hyacinthi* in a fluid consisting of 1 part of strongly alkaline beef broth (286b) in 500 parts of distilled water. *Ps. campestris* and *Ps. phaseoli* also clouded this fluid. These cultures were made in clean tubes of resistant glass.

(2) *Ps. hyacinthi* grew readily in distilled water containing 1 to 2 per cent of Witte's peptonum siccum, and the precipitate was yellow. Growth in 1 per cent peptone water in the open end of fermentation tubes, as we have already seen, was increased by the addition of 1 per cent doses of grape sugar, fruit sugar, cane sugar, or dextrin, and was not perceptibly increased by the addition of 1 per cent doses of milk sugar, maltose, mannitol, or glycerol. If under these conditions any acid was formed from any of these substances, it was overlooked or obscured by the alkali.

(3) In distilled water (10 c. c. portions in tubes of resistant glass) containing 4 per cent of Witte's peptonum siccum and 4 per cent of dextrin there was little or no retardation of growth. On the twelfth day the fluid was plainly alkaline to litmus. On the twenty-ninth day there was an abundant yellow rim and a very copious dull-yellow precipitate (6 mm. deep). The cloudy fluid was plainly and rather strongly alkaline. On this date there was several times as much precipitate as in the corresponding tubes of *Ps. campestris* and *Ps. phaseoli*. On the fortieth day the fluid was strongly alkaline. It was still cloudy with rolling clouds on shaking, and there was no brown stain in it. On the sixty-fifth day the fluid was moderately alkaline. No crystals

were present, and a feeble brown stain, thought to have been detected on the fifty-fourth day, was not well enough developed to be recorded as certainly present. On this date there was more than twice as much precipitate as in the corresponding tube of *Ps. campestris.* This dextrin had been ten times precipitated with alcohol in the Division of Chemistry, United States Department of Agriculture. It gave a heavy yellowish precipitate on boiling 1 minute in Soxhlet's solution, but no precipitate on boiling 2 minutes in Barfoed's reagent.

(4) In distilled water (10 c. c. in tubes of resistant glass) containing 4 per cent of Witte's peptonum siccum and 4 per cent of maltose there was no retardation of growth, and for the first week or so the culture closely resembled the preceding. On the twelfth day the fluid was distinctly alkaline to litmus, but it was less cloudy than the preceding and there was far less precipitate. On the twenty-ninth day the fluid was plainly and rather strongly alkaline, but there was only about one-tenth as much precipitate as in the tube containing the dextrin. On the fortieth day the fluid was still cloudy, but was not browned. The rim was not very abundant and was paler than in the preceding. The precipitate was the same shade of pale yellow as in the tube containing the dextrin, but there was only one-tenth to one-fifteenth as much. On the sixty-fifth day the fluid was strongly alkaline, but both in this and in the preceding the blue color soon disappeared from the neutral litmus paper, leaving it redder than before. No crystals were formed.

In the corresponding tube of *Ps. campestris* there was a distinct browning of the fluid, which was first noticed on the fortieth day. *Ps. phaseoli* browned neither fluid.

CRUDE VEGETABLE SUBSTANCES.

The behavior of *Ps. hyacinthi* in contact with steam sterilized solids and fluids derived from plants has been discussed so fully under Sensitiveness to acids and Growth on solid media that it is only necessary here to recapitulate a few of the more important discoveries.

(1) All my observations tend to show that plant acids, even in comparatively small doses, prevent growth, and that still smaller quantities retard growth. It is, therefore, probable that these acids do not serve directly as food. Certainly the behavior of this organism in nutrient fluids containing malic acid is extremely unlike that of organisms which are believed to use this acid as a food, e. g. *Bacillus amylovorus.*

(2) Starch, as we have seen, is transformed into substances which can be assimilated only with the greatest difficulty.

(3) Growth on steamed vegetables poor in sugar was always rather meager. Substrata containing rather more sugar gave a correspondingly better growth.

(4) Growth on vegetables rich in grape sugar or cane sugar was copious and long continued. These two sugars are excellent foods, and when not present in such excess as to inhibit growth (probably by plasmolysis) they greatly favor the multiplication of this organism.

SUGAR GELATIN.

(See Growth on solid media.)

SUGAR AGARS.

Some interesting results were obtained by adding large doses of sugar to 10 c. c. portions of Mr. Dorsett's +15.5 meat-extract peptone agar (see Growth on solid media) and growing on it the various yellow organism in slant cultures. Their behavior on these media was always compared with that on check tubes of the sugar-free agar.

NINE PER CENT SUGARS.

First series.

Agar recently tubed and slanted (10 cc. to 1 gram of the sugar). Inoculations with *Ps. hyacinthi*, using bright-yellow slime from a starch jelly culture 28 days old. All the inoculations were made in the same way and with approximately the same amount of material.

Third day.

(1) *Check.*—Streak 2 by 75 mm., distinct the whole length of the track, best developed at the lower end, where it is distinctly pale yellow. In the middle 3 c. m. it consists of separate colonies.

(2) *Grape sugar (1 gram of Merck's c. p. anhydrous).*—Streak invisible except in a very favorable light, where it looks like a colorless film.

(3) *Cane sugar (1 gram of white commercial).*—A thin pale yellow growth over the whole slant. In strong contrast with the grape-sugar agar. Also more growth than in the check tube.

Seventh day.

(1) *Check.*—The streak is now 3 to 5 mm. wide. All of the colonies have fused into a smooth, yellow, wet-shining homogeneous surface.

(2) *Grape sugar.*—Growth mostly in the form of separate colonies and less than in the check tube—i. e., a distinct retardation. There are many of these colonies, and where they have coalesced the color is about the same shade of yellow as in the check tube.

(3) *Cane sugar.*—The whole surface of the slant agar is covered and hidden by a copious pale yellow growth. Six times as much growth as in the check tube and 8 or 10 times as much as on the grape-sugar agar.

Sixteenth day.

(1) *Check.*—The streak has not widened any. It is smooth, translucent, wet-shining, and distinctly pale yellow. The margins of the streak are thin but distinct. A penholder is plainly visible under the streak.

(2) *Grape sugar.*—Growth has quadrupled and is now about 3 times as abundant as in the check tube, but its surface is very unlike that of the latter. The surface,

which is pale yellow, has a peculiar roughened or areolate appearance, which appears to be due to wrinkles extending in various directions. The shallow pits are 2 to 3 mm. in diameter.

(3) *Cane sugar.*—Fully 6 times as much growth as in the check tube. The penholder can not be seen under it. Color pale yellow, a little paler than in the check tube. Surface not smooth as in the check tube nor wrinkled as on the grape-sugar agar, but finely roughened.

Twenty-ninth day.

(1) *Check.*—Little change. The streak is 3 to 6 mm. wide. Its surface is smooth and wet-shining, and to either side, on the lower part of the slant, there is a slight chemical whitening of the surface of the agar. No brown stain.

(2) *Grape sugar.*—About 4 times as much growth as in the check tube. The bacterial layer covers all but the upper part of the slant and there is some growth between the agar and the walls of the tube. Growth the same shade of yellow as in the check tube, or only a trifle paler. No brown stain. No whitish chemical film on the agar beyond the streak. Surface wet, but not smooth as in the check tube. The extreme upper part of the streak is still composed of separate colonies, and the rest of it is areolated i. e., covered with tiny ridges and depressions.

(3) *Cane sugar.*—Color uniformly pale yellow. Surface drier than it was and slightly roughened, but not coarsely areolate, as on the grape sugar agar. Streak less translucent than in the check tube, i. e. almost opaque. No brown stain. The culture has a feeble smell. On boiling the contents of this tube for one minute in Soxhlet's solution there was a very heavy precipitate of copper oxide. Sugar and agar had both been tested for reducing substances previous to inoculation and neither one gave any trace of copper oxide on boiling two minutes in Soxhlet. The slime remaining in the tube was very feebly alkaline to litmus, i. e., much less alkaline than might have been expected from the amount of growth. This is presumptive evidence that most of the alkali had been neutralized by some acid.

Forty-seventh day.

(1) *Check.*—The streak is drying out. It is 65 by 3 to 6 mm., i. e., it has spread but little. It is still smooth, wet-shining, and so translucent that a penholder can be seen through it. The streak has well-defined margins, beyond which the surface is feebly whitened. On neutral litmus paper the saffron-yellow slime has an alkaline reaction. Examined microscopically, this slime consists of zooglœæ and short, slender rods, single or in pairs. Rods in fours are rare, and chains are short and exceedingly rare.

(2) *Grape sugar.*—The bacterial layer is gallstone yellow. It now covers almost the entire slant (70 by 16 mm.), and is about 20 times as abundant as in the check tube. It scrapes off easily and gives an acid reaction on neutral litmus paper. A few separate colonies persist on the upper dried-out part. The surface is not smooth, but roughened, and wrinkled slightly in the lower part of the slant. Examined microscopically, the slime consists of zooglœæ, chains, and short, slender rods, single, in pairs, or in fours. Chains of 10 to 20 or more segments are numerous. In some the individual segments are easily discernible, in others not. Apparently some of the rods are motile. No spores.

(3) *Cane sugar (another tube of the same age, but containing only 6.75 per cent of sugar).*—Growth dense and finely roughened (fine wrinkles under the hand lens). No brown stain. No crystals. No chemical film. At least 10 times as much growth as in a check tube. Slime, buff yellow (R. VI–19), acid to neutral litmus paper. Examined microscopically, the slime consists of zooglœæ, numerous chains of 10 to 40 segments, and many single rods, pairs, and fours joined end to end. In many of

the chains, but not all, the individual elements are visible. No spores. The microscopic appearance closely resembles that of the slime from the grape-sugar agar, the principal difference being the tendency to longer chains or filaments.

Second series.

The check tube had the driest surface; the surface of the fruit-sugar agar was the moistest. Inoculations from a slant-agar culture of *Ps. hyacinthi* 13 days old. All made in the same way and with approximately the same amount of material.

Third day.

(1) *Check.*—Streak 78 by 5 to 12 mm., pale yellow, translucent, smooth, wet-shining, homogeneous looking, and not scanty, i. e., a good growth over the whole length of the slant.

(2) *Fruit sugar (1 gram of Schering's diabetine).*—No growth, although inoculated just as copiously.

(3) *Grape sugar (1 gram of Merck's c. p. anhydrous).*—A feeble growth consisting of scattered colonies which, in some places, have fused into a very thin layer. Not one-twenty-fifth as much growth as in the check tube. Grape sugar in 9 per cent doses distinctly retards growth. (This growth doubled during the next 24 hours.)

Fifth day.

(1) *Check.*—Much as before.

(2) *Fruit sugar.*—No growth.

(3) *Grape sugar.*—There is now nearly as much growth as in the check tube. The lower one-half of the slant is covered, and the upper one-half bears scattering yellow colonies. The surface is not smooth, as in the check tube, but is distinctly shagreened to the naked eye. The yellow slime is very feebly alkaline, inducing only the barest trace of blue on wet or dry neutral litmus paper.

Eighteenth day.

(1) *Check.*—A thin, smooth, moist, pale-yellow slime covers nearly the entire slant. There is no brown stain in the agar.

(2) *Fruit sugar.*—No growth. Fragments of the moist agar pressed on neutral litmus paper redden it.

(3) *Grape sugar.*—A copious, pale-yellow, coarsely wrinkled growth now covers the whole slant. This layer scrapes off easily, and is very feebly alkaline to neutral litmus paper. There is no brown stain in the agar.

Fifty-third day.

(1) *Check.*—Slime feebly alkaline.

(2) *Fruit sugar.*—No growth. Failure to grow was attributed to the restraining influence of lactic acid put into this sugar by the manufacturers to improve its keeping qualities.

(3) *Grape sugar.*—Streak somewhat wrinkled and on the margins slightly areolate. Slime now distinctly acid to neutral litmus paper, no trace of any alkaline reaction. Culture diluted (shaken) with 40 c. c. of distilled water and retested. It is now neutral or only very feebly acid. On boiling this water a little acid is given off in the first vapors (CO_2?), but less than from a corresponding culture of *Ps. campestris.* On concentrating this fluid by continued boiling it became plainly more acid to litmus paper, indicating the presence of a small amount of some non-volatile acid. Cultures of *Ps. campestris* behaved in the same way.

RENEWED EXPERIMENTS WITH FRUCTOSE.

The fructose (Schering's diabetine) was first titrated with caustic soda and litmus to determine its acidity. This was such that 10 c. c. of $\frac{N}{10}$ NaOH were required to render 10 grams moderately alkaline to litmus. One-half c. c. of this thick alkaline sirup was then pipetted into 7 c. c. of Dorsett's agar for one experiment and 1 c. c. of the sirup into 10 c. c. of the agar for another experiment. The agar was then resterilized and slanted in the usual way. The check tubes had been slanted longer than the others and their surface was somewhat dry. All were inoculated with *Ps. hyacinthi* from a slant agar culture 24 days old, in the same way and with approximately the same amount of material.

Second day.

(1) *Check.*—A feeble growth in the form of scattered colonies.

(2) *Fruit sugar (one-half c. c. sirup).*—A feeble growth, which was visible sooner than in the check tube, i. e., within 18 hours.

(3) *Fruit sugar (1 c. c. sirup).*—A very slight growth, not one-fourth as much as in the preceding.

Fourth day.

(1) *Check.*—Not a good growth. It occurs colony-wise over the streak. This agar had been slanted a long time and the surface was becoming too dry for good growth.

(2) *Fruit sugar (one-half c. c. sirup).*—A distinct multiplication during the last 48 hours, but not yet a homogeneous streak, i. e., growth thin in some places and more abundant in others. Not yet more growth than would have appeared in the same time on a freshly slanted check tube.

(3) *Fruit sugar (1 c. c. sirup).*—Very little growth, i. e., not one-twentieth as much as in the preceding. This substratum evidently retards growth.

Seventh day.

(1) *Check.*—A much better growth. The colonies touch or nearly touch, forming a thin, distinctly yellow slime over nearly the whole slant.

(2) *Fruit sugar (one-half c. c. sirup).*—There is now more growth than the agar alone would give. The streak is dense and rather abundant (47 by 10 mm.), pale yellow, smooth, and wet-shining.

(3) *Fruit sugar (1 c. c. sirup).*—Growth very feeble. There has been a slight increase during the last 3 days, but the growth is not now one one-hundredth, perhaps not one one-hundred-and-fiftieth, as much as in the preceding tube.

Twelfth day.

(1) *Check.*—The colonies, for the most part, have now fused into a smooth surface.

(2) *Fruit sugar (one-half c. c. sirup).*—A very copious, pale yellow, smooth, wet-shining growth covers the whole slant, and is growing in between the tube and the agar. At least 4 times as much growth as in the check tube.

(3) *Fruit sugar (1 c. c. sirup).*—The restraining influence is being overcome. About one-third as much growth as in the preceding, and excellent where it has obtained a foothold. This growth is of the same character as in the preceding.

Sixteenth day.

(1) *Check.*—Growth decidedly yellow, still thin.

(2) *Fruit sugar* (*one-half c. c. sirup*).—Growth has continued. It is wet-shining, very smooth, and extremely copious. About 10 times as much growth as in the check tube. Fructose distinctly favors growth unless all of this excess is attributable to the sodium lactate formed by neutralizing the lactic acid, which is extremely improbable.

(3) *Fruit sugar* (*1 c. c. sirup*).—A marked increase of growth during the last 4 days. A considerable part of the slant which was then free is now covered. The slime is pale yellow; the surface is very smooth and wet-shining.

Thirtieth day.

(1) *Check.*—Surface so dry that not all of the colonies have fused. No crystals. No stain of the agar.

(2) *Fruit sugar* (*one-half c. c. sirup*).—The pale yellow, wet-shining, smooth slime is 3 mm. deep over the whole surface of the slant. The color is dull yellow, but there is no reason for thinking it contaminated. No brown stain. No crystals in the agar. Growth has been enormously stimulated by this sugar.

(3) *Fruit sugar* (*1 c. c. sirup*).—The entire surface of the slant (15 by 53 mm.) is now covered with a pale yellow, smooth, very wet-shining slime. There is no brown stain, and there are no crystals in the agar.

SEVENTEEN PER CENT SUGARS.

Mr. Dorsett's +15.5 sugar-free agar was also the basis of all of these tests. Each tube contained exactly 10 c. c. of agar to which was added 2 grams of the sugar to be tested. The slant surfaces were all inoculated in the same manner, and with approximately the same amount of material, viz, loops of bright-yellow slime from a coconut culture 8 days old.

First day (22 hours at 27° to 30° C.).

(1) *Check.*—A distinct, wide, pale yellow streak.

(2) *Grape sugar* (*2 gr. Merck's c. p. anhydrous*).—Streak not visible.

(3) *Cane sugar* (*2 gr. white commercial*).—A meager growth. One-tenth to one-twentieth as much as in the check tube. For the most part, the streak is invisible and nowhere shows more than a trace of growth.

Fourth day (temp. 27° to 31°).

(1) *Check.*—Streak smooth, wet-shining and rather bright yellow, but not dense enough to be opaque. It is 72 by 5 to 6 mm. The margins of the streak are distinct and there is no whitish efflorescence on the surface of the agar around the streak.

(2) *Grape sugar.*—Doubtful. No visible growth except in very favorable lights. If any growth at all, not one one-hundredth as much as in the check tube. There can be no doubt that 17 per cent grape-sugar agar exerts a very distinct retarding influence on *Ps. hyacinthi.*

(3) *Cane sugar.*—A well-developed streak 62 by 5 to 8 mm. It appears to be as dense as in the check tube, but is paler yellow, i. e., the color is exactly that of a 4 days' growth of *Ps. campestris* on the check agar. This tube and the check tube are in marked contrast with the preceding.

Eighth day.

(1) *Check.*—The streak has thickened a little, but has not widened.

(2) *Grape sugar.*—What looked on the fourth day like mere dried-out portions of the slime used in making the inoculation has now developed as a distinct growth in

two places, aggregating 2 square cm. Where the organism has secured a foothold the slime is distinctly pale yellow, but much of the part which was streaked bears no growth whatever, and altogether there is not one-tenth as much growth as in the check tube. The surface of this slime is not smooth, as in the check tube, but is minutely fissured and roughened all over.

(3) *Cane sugar.*—As much growth as in the check tube, but paler yellow. Thus far the sugar has not stimulated growth. The streak is now 5 to 9 mm. wide. It has thickened some since the last record, but has not widened much.

Thirteenth day.

(1) *Check.*—Streak well developed, smooth, wet-shining, and distinctly yellow. The margins are well defined, and the body of the streak is not opaque, i. e., the penholder can still be seen through it. It shows very little tendency to spread, i. e., it is still only 5 to 7 mm. wide. There are no projections from the under side of the streak into the agar (such growths appeared in case of an undescribed, white, spore-bearing organism, derived from rotting tomato fruits, and grown on this same agar). There is now a slight but distinct bloom (chemical whitening) on the surface of the agar beyond the streak.

(2) *Grape sugar.*—About one-third, or possibly one-half as much growth as in the check tube. The color is the same, but the surface appearance is very different. The wet surface is not smooth, but is roughened, or areolated, as if made up of fused zooglœæ with grooves between them. There is no chemical whitening of the surface of the agar beyond the streak.

(3) *Cane sugar.*—Streak mostly 6 to 10 mm. wide. Surface drier and paler yellow than in the preceding or in the check tube. No growths into the agar from the under surface of the streak. Seventeen per cent cane-sugar agar is not nearly so favorable to the growth of this organism as 9 per cent. There is now but little more growth than in the check tube, whereas on the 9 per cent cane-sugar agar there was 6 times as much growth in one-half this time, the temperature in both cases being approximately the same, i. e., near the optimum.

Seventeenth day.

(1) *Check.*—The yellow slime is plainly alkaline to good neutral litmus paper, much more so than that on the grape-sugar or cane-sugar agar.

(2) *Grape sugar.*—Slime neutral or very slightly alkaline.

(3) *Cane sugar.*—Slime neutral or only very slightly alkaline.

Thirtieth day.

(1) *Check.*—The streak begins to dry out. Its surface is smooth. There has been no widening. Beyond the streak the whitish chemical film remains, but is not very pronounced. No brown stain. No crystals.

(2) *Grape sugar.*—A pale yellow well-developed streak (50 by 5 to 9 mm.). It has not spread widely, and is still translucent. The surface is rather coarsely roughened and looks as if many large zooglœæ had fused, leaving grooves between each one. The surface of the individual hummocks is smooth, wet-shining, firm, elastic, and scrapes off only after the use of considerable force. Examined under the microscope, this growth consists of slender rods mixed in with some chains. The rods are single, in pairs, and in fours joined end to end. There is no brown stain.

(3) *Cane sugar.*—The growth is now two or three times as abundant as in the check tube. It is very dense, especially on the lower part of the slant, where it is crowded up into high folds. The upper part shows lesser wrinkles. No brown stain and no chemical film on the clear agar to either side of the streak. The slime is pale yellow

and very feebly alkaline. It is made up of small roundish zooglœæ, short chains of a dozen or more segments, and slender short rods, single, in pairs, or fours. Some of the rods are actively motile.

Sixty-sixth day.

(1) *Check.*—Slime strongly alkaline to neutral litmus.

(2) *Grape sugar.*—Slime not alkaline. Distinctly acid on neutral litmus paper.

(3) *Cane sugar.*—No alkaline reaction. Slime distinctly acid on neutral litmus paper.

TWENTY-THREE PER CENT SUGARS.[1]

These cultures were like the preceding except that for each 10 c. c. of agar 3 grams of the specified sugar was used. The check tubes had been slanted longer than the others and their surface was drier. All were smeared with *Ps. hyacinthi* from an agar culture 24 days old in the same way and with approximately the same amount of material. The alkaline fruit-sugar agars already described were inoculated at the same time and from this same culture, which was the check tube described under the 17 per cent sugar agars.

Seventh day.

(1) *Check.*—A thin distinctly yellow growth over nearly the whole slant.

(2) *Grape sugar (3 grams Merck's c. p. anhydrous).*—No growth.

(3) *Cane sugar (3 grams white commercial).*—A very feeble, scrappy growth, not forming a streak, but confined to the immediate vicinity of some small fragments of slime, which were left unspread when the agar was inoculated. Not more than two or three times as much slime present as was put into the tube in making the inoculation. Twenty-three per cent cane-sugar agar strongly retards growth.

Twelfth day.

(1) *Check.*—Fully twice as much growth as on the cane-sugar agar.

(2) *Grape sugar.*—No growth, although the surface of the entire slant was rubbed with a mass of yellow slime as large as a pin head.

(3) *Cane sugar.*—A distinct, rather thin, wet, yellow, rough-surfaced growth, which covers about one-half of the slant (lower half).

Thirtieth day.

(1) *Check.*—Surface of the streak smooth, wet-shining, and distinctly yellow; no reticulations or shagreen.

(2) *Grape sugar.*—No growth. *Ps. hyacinthi* will not grow on 23 per cent grape-sugar agar.

(3) *Cane sugar.*—The lower three-fourths of the slant is covered with a distinctly yellow growth, which is rather dry, but looks wet under the hand lens. The surface is not smooth, but is reticulate, areolate, or shagreened, the portions between the grooves being lighter yellow and very smooth. This areolation is shown in Bulletin 26 of this Division, in text fig. 3, which was made from this culture on the thirty-third day. The agar has not dried out much, but the slime shows no tendency to flow.

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[1] The expressions 9, 17 and 23 per cent are used for convenience. Of course, the writer is aware that 3 grams added to 10 c. c. does not make exactly 23 per cent.

Thirty-seventh day.

(1) *Check.*—No record.

(2) *Grape sugar.*—No record.

(3) *Cane sugar.* —No crystals. No stain in the agar. The bacterial layer peels off easily in fragments, leaving a smooth, clean agar surface. This layer is not sticky or elastic, and dissolves with difficulty in water, breaking up into rather coarse fragments. Examined under the microscope, it consists of zooglœæ, single rods, doublets, and chains. The latter are 50 to 100 μ. long.

SUMMARY OF RESULTS WITH SUGAR AGARS.

(1) *Ps. hyacinthi* grew without retardation on the check tubes, and the surface was always smooth.

(2) Addition of 9 per cent grape sugar retarded growth. Finally growth was more abundant than in the check tubes, and the surface was areolated.

(3) Addition of 17 per cent grape sugar retarded growth for a longer time. This was finally more abundant than in the check tubes, and its surface was areolated.

(4) Addition of 23 per cent grape sugar entirely prevented growth.

(5) Addition of 9 per cent cane sugar did not retard growth, and after a few days greatly stimulated it. The surface was wrinkled or finely roughened.

(6) Addition of 17 per cent cane sugar retarded growth. This finally became more copious than in the check tube, but it was never as abundant as on the 9 per cent cane-sugar agar. The surface was wrinkled.

(7) Addition of 23 per cent cane-sugar agar retarded growth for a longer time, but did not prevent it. The surface was areolated or shagreened.

(8) Addition of 9 per cent acid fructose (Schering's diabetine) entirely prevented growth. When the lactic acid was neutralized by caustic soda growth ensued, but was retarded for some time. In the end it was very abundant.

Some interesting comparisons were obtained from concomitant cultures of *Ps. campestris*, *Ps. phaseoli*, and *Ps. stewarti*.

(1) On the check or sugar-free agar all three grew without retardation, and did as well as *Ps. hyacinthi*. This agar was not stained brown and no crystals were formed, but the superficial white chemical film appeared whichever organism was used. This film also failed to appear on the sugar agars, whichever germ was used. In the check tubes of each the slime was feebly alkaline at first and finally became strongly alkaline. On the contrary, with grape sugar or cane sugar, the reaction of the slime changed very slowly from alkaline to acid, whichever organism was used. All four invert cane sugar. All are alike in producing a small amount of non-volatile acid when grown on

this agar in the presence of grape sugar or cane sugar. All were much alike in color, but frequently the hyacinth germ was the brighter yellow.

(2) The growth of *Ps. campestris*, *Ps. phaseoli*, and *Ps. stewarti* was not retarded by 9 per cent grape sugar. On the contrary, it was stimulated from the very start. At the end of the first 48 hours on this agar *Ps. campestris* showed about twice as much growth, *Ps. phaseoli* "more growth," and *Ps. stewarti* four times as much growth as in the corresponding check tubes. On the seventh day *Ps. campestris* showed ten times as much growth as *Ps. hyacinthi*, and three times as much as in its own check tube (ten times as much on the sixteenth day). On this date *Ps. phaseoli* had made twice as much growth as in the check tube (ten times as much on the sixteenth day). On the same date *Ps. stewarti* had made at least five times as much growth as in the check tube.

In a second series of experiments with this agar *Ps. campestris* showed, on the third day, twice as much growth and *Ps. phaseoli* two and one-half times as much as there was in the check tubes. There was no retardation whatever.

(3) Addition of 17 per cent grape sugar retarded the growth of *Ps. campestris* and *Ps. phaseoli* (*Ps. stewarti* was not tried), but they overcame the injurious influence sooner than *Ps. hyacinthi*. If the volume of growth of *Ps. hyacinthi* on this agar on the sixth day be taken as 1, then that of *Ps. campestris* was 10 and that of *Ps. phaseoli* was 15 to 18.

(4) Addition of 23 per cent grape sugar entirely prevented the growth of *Ps. phaseoli* and seriously retarded that of *Ps. campestris*, but did not prevent it. On the contrary, when the retarding influence was overcome growth was greatly stimulated. On the seventh day this growth was only about one-fifteenth as much as in the check tube, or as on the 23 per cent cane-sugar agar. On the sixteenth day there was a marked increase of growth, but there was not one one-hundredth as much as in the corresponding tube of cane-sugar agar. On the thirtieth day the streak was 23 by 6 to 8 mm. On the thirty-seventh day growth had doubled, the streak now being 40 by 3 to 12 mm. The slime dissolved readily in water and consisted largely of chains 50 to 100 μ long. In a repetition of this series of experiments, 23 per cent grape sugar retarded but did not prevent the growth of *Ps. phaseoli*. The surface was rubbed with loops from agar cultures, but growth did not appear until the fourth day, and then only colony-wise.

(5) On the 9 per cent (acid) fructose agar *Ps. phaseoli* refused to grow. *Ps. campestris* obtained a precarious foothold, but grew only a little.

(6) Addition of 17 or 23 per cent cane sugar did not retard the growth of *Ps. campestris* or *Ps. phaseoli*, at least, not to any notice-

able extent. On the contrary, within a few days growth was enormously stimulated. If the volume of growth of *Ps. hyacinthi* on the 17 per cent cane-sugar agar at the end of eight days be reckoned as 1, then that of *Ps. campestris* was 2 or 3 and that of *Ps. phaseoli* was 3 or 4. On the 23 per cent cane-sugar agar, on the fourth day, the growth of *Ps. campestris* was five times as much as in the check tube, and that of *Ps. phaseoli* "vastly better." On the twelfth day the cultures of *Ps. campestris* and *Ps. phaseoli* resembled each other closely in color, general appearance, and amount of growth, which latter was ten times that in the corresponding tube of *Ps. hyacinthi*. On the thirty-sixth day the slime of *Ps. phaseoli* consisted of rods. doublets, fours, and many chains 50 to 120 μ long.

(7) The growth of *Ps. campestris* and *Ps. phaseoli* on the sugar agars was smooth, wet-shining, and often abundant enough and thin enough to flow like thick sirup on tilting the tubes. That of *Ps. hyacinthi* would never flow and was distinctly areolated, reticulated, wrinkled, or shagreened, as already described.

SODIUM ACETATE.

The stock (495) containing this substance was compounded as follows:

Distilled water, 400 c. c.
Dipotassium phosphate, 0.800 gram.
Magnesium sulphate, 0.040 gram.
Ammonium phosphate, 0.040 gram.
Sodium acetate, 2 grams.

This medium was filled into cotton-plugged test tubes and sterilized in the usual way. It was inoculated with *Ps. hyacinthi* very copiously from a young culture on coconut. It was under observation 5 weeks at 25° to 30° C., but growth progressed very slowly and was never anything more than feeble. At the end of the 5 weeks the fluid was still feebly clouded and there was no rim of germs or pellicle, but in the fluid on the wall of the tube were several hundred small, ragged, whitish flocks and on the bottom there was a pale yellow precipitate 5 mm. wide. The growth was not better than in Uschinsky's solution.

Ps. campestris also grew feebly in this fluid, and *Ps. stewarti* would not grow at all (only one test).

NUTRIENT STARCH JELLY WITH SUGARS, GUMS, AND ALCOHOLS.

Some comparative tests of these four yellow organisms as to color, rate of growth, etc., were made in tubes of slant nutrient, starch jelly to which 500 milligrams of special kinds of carbon foods were added—e. g., dextrin, lactose, maltose, etc. The growth in these tubes was compared with that in tubes of starch jelly to which the sugars, etc., were not added. My general conclusions are as follows:

Table V.—*Behavior of Ps. hyacinthi, etc., on nutrient starch jelly with various carbon foods.*

Substance added.	Organisms.			
	Ps. hyacinthi.	Ps. campestris.	Ps. phaseoli.	Ps. stewarti.
Lactose....	....	....	A marked stimulating effect. Growth very copious, sirupy and with a magnificent production of the yellow pigment.	No increased growth.
Dextrin	A stimulating effect. Bright yellow. Growth several times as abundant as in the check tubes. Not sirupy.	....	....	No increased growth.
Maltose....	Feeble at first, then several times as much as on check. Bright yellow.	....	....	No stimulating effect. Very feeble pale-yellow growth.
Galactose..	A stimulating effect. Several times as much growth as in check. Slime very bright yellow.	....	....	Marked stimulating effect. As much growth or nearly as much as on cane sugar. 100 times as much growth as on the glycerine jelly.
Cane sugar	Copious bright-yellow growth. Several times as abundant as in check. After 128 days the starch immediately under the bacterial layer gave a marked reaction with iodine, blue and purple. Litmus reaction feebly acid.	Copious, smooth, wet-shining pale-yellow growth. Not much more abundant and not so yellow as finally in the glycerinated jelly.	....	Marked stimulating effect. A copious, smooth, wet-shining, buff-yellow, sirupy growth.
Mannit....	No stimulating effect. Not more growth than in the check tubes.	....	....	No stimulating effect.
Glycerin...	Growth feeble for some weeks as if retarded, color pale. After 128 days the whole surface of the slant (7 sq. cm.) was covered with a dense growth. The color was a uniform dull yellow, a little brighter than wax yellow. The entire surface was shagreened. The starch was not browned. It had lifted a little from the bottom on which was a small amount of yellow fluid due to the solvent action of the glycerol. Neither slime nor fluid were alkaline, both appeared to be neutral when wet, and the litmus was only slightly acid when dry. The slime was not sticky. The starch even immediately under the yellow layer reacted at once blue or purple with iodine.	Retardation of growth, of yellow pigment, and of diastasic action. After 24 days, however, a copious, sirupy smooth, wet-shining, rather bright yellow growth over whole slant, and culture then in marked contrast to *Ps. phaseoli* and *Ps. stewarti*.	Retards growth, diastasic action, and formation of yellow pigment (only one test). After 24 days not one one-hundredth as much growfh as in the check or the lactose jelly and no distinct yellow pigmentation.	No stimulating effect, and apparently a distinct retardation.

TEMPERATURE EXPERIMENTS.

Thermal Death Point.

Some difficulty was experienced in determining accurately the thermal death point of *Ps. hyacinthi* owing to the slight variability in sensitiveness of individual rods. Considerable trouble was also experienced for some time owing to the frequent unaccountable failure of the germs to grow in some of the fluid cultures (see Sensitiveness to acids).

Most of these experiments were made in thin-walled test tubes 16 to 17 mm. in diameter, and containing exactly 10 c. c. of fluid (usually beef broth) entirely free from any trace of sediment or cloudiness. These tubes were inoculated in each case with big loops from fluid cultures only a few days old (1 to 11), and great care was taken in making the inoculations not to wet the walls of the tube above the fluid, and also to keep the tubes upright from first to last. The exposures were made by plunging the inoculated tubes into a hot-water bath nearly to their top, and keeping them in it at the given temperature for exactly 10 minutes. They were then removed, and either cooled quickly under running water or left to slowly acquire the temperature of the room. Duplicate tubes were always inoculated and maintained at the living-room temperatures for comparison. On two occasions poured plates were also made, using a large quantity of the culture fluid so as to determine more precisely the proportion of the germs killed by the heating.

The hot-water bath employed was the Ostwald-Pfeffer, using a very sensitive Roux metal-bar thermo-regulator, and a stream of compressed air for the motive power. The thermometer employed was a very sensitive one, belonging to a set made by Max Kaehler and Martini, of Berlin, and compared with the standard hydrogen thermometer of the International Bureau of Weights and Measures, Washington, D. C. With this apparatus, which keeps the water uniformly in motion, it was easy to maintain approximately constant temperatures for short periods.

The following is a detailed account of these experiments:

I. December 3: One tube of stock 204 inoculated with a large loop from tube 6 December 1. This tube was allowed to stand 1 hour and then plunged for 10 minutes into water at 54.30° C. Cooled at room temperature. Result: Under observation several weeks, but no growth.

II. December 3: One tube of stock 204 inoculated with a large loop from tube 6 December 1. This tube was allowed to remain 3 hours at room temperatures and then plunged for 10 minutes into water at 49.80° C. Cooled at room temperatures. Result: No growth. Tube under observation several weeks.

III. December 3, 1896: One tube of stock 204 (1:2 acid beef broth, i. e., no peptone or alkali added) inoculated with a large loop from tube 6 December 1, which was a well-clouded,[1] 43-hour culture in stock 204. The germs were allowed to grow in the broth 1 hour, after which the tube was plunged for 10 minutes into water at 46° C. and then cooled at room temperatures. Result: Tube under observation several weeks, but no growth.

Check.—On December 3, at the same time as I, II, and III, another tube of stock 204 was inoculated with a large loop from tube 6, December 1, and left at room temperatures. Result: Growth was retarded, but not prevented. On the eighth day the medium was still clear, but on the thirteenth day the fluid was faintly clouded with a little precipitate and with good rolling clouds on shaking. This broth was not titrated, and consequently its grade of acidity was not known. It was feebly acid to litmus and contained a small amount of muscle sugar.

IV. December 8: Two tubes of stock 204, one inoculated from tube 6, December 1, and the other from tube 7, December 1 (a beef-broth culture inoculated with descendants of germs isolated from another hyacinth bulb). Each tube received a large loop of the fluid, and as the cultures were some days older, more germs than tubes I, II, III, and their check. After 1 hour both tubes were plunged for 10 minutes into water at 43.25° C., and then cooled at room temperatures. Result: December 11 both tubes are faintly clouded; December 17, moderately cloudy with rolling clouds on shaking and a small amount of yellow precipitate. The germs are not killed by 43° C., and are little, if any, retarded, the two check tubes clouding in about the same time and manner.

V. December 8: Two tubes of stock 204, one inoculated from tube 6, December 1, and the other from tube 7, December 1. In all respects a duplicate of IV, except that the water bath was 44.35° C. The tubes were cooled at room temperatures. Result: December 11 both tubes are faintly clouded; December 17, no pellicle, but a moderate amount of yellow precipitate and a good many small, roundish zooglœæ in the top layers of the fluid. These zooglœæ diffuse through the fluid on gentle shaking. Temperature of 44.35° C. does not kill or much retard growth. These tubes were compared with the 2 check tubes mentioned under IV.

VI. December 8: Two tubes of stock 204, one inoculated from tube 6, December 1, and the other from tube 7, December 1. In all respects like V, except temperature of water bath, which was 45.20° C. The tubes were cooled at room temperatures. Result: December 11, both tubes perfectly clear. On December 17, when next examined, the fluid in each tube was moderately cloudy, with distinct rolling clouds on shaking. Cloudiness easily visible without shaking. A little precipitate. Temperature of 45.20° C. does not kill, but considerably retards growth, the 2 check tubes (those mentioned under IV) being cloudy on December 11. The germs in tubes 6 and 7, December 1, were derived (as already stated) from different hyacinth bulbs.

VII. May 14: Six tubes of stock 217 (cauliflower broth feebly alkaline to neutral litmus), each inoculated with a loop from tube 5, May 10 (stock 218, a potato broth which came up slowly and was moderately cloudy, with rolling clouds on shaking). Four of these tubes were plunged for 10 minutes into water at 45.60° C., and two were held as checks. Result: The tubes were under observation for several weeks, but all of them, including the two checks, remained sterile. There was no apparent reason for the failure of the two checks, since the material used for inoculation was living (see VIII), and closely related organisms grew well in this broth, e. g., *Ps. phaseoli*.

VIII. On May 14, from two tubes of litmus neutral beef broth peptone agar, two poured plates were prepared in the following manner:

(1) One cubic centimeter of the well-clouded potato-broth culture (tube 5, May

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[1] It is possible that part of this clouding may have been due to dead or feeble individuals derived from the original inoculation, which was probably from a solid culture.

10) was transferred by means of a sterile pipette to 10 c. c. of fluid agar (cooled to 41° C.), and after thorough shaking was poured into a sterile Petri dish.

(2) The remainder of the culture was then plunged for 10 minutes into water at 46.05° C.; it was then cooled at room temperatures for a few minutes and 1 c. c. taken out by means of another sterile pipette, put into another tube of melted agar (10 c. c. at 41° C.), and when thoroughly shaken poured into a second sterile Petri dish.

These two dishes were then kept at living-room temperatures and compared from time to time by turning them bottom up under the microscope. Result: May 18 (1) agar uniformly milky cloudy. Under the microscope innumerable small colonies are to be seen. Number of colonies estimated at 8,000 to 10,000 per field (Zeiss 16 mm. and 12 comp. oc.) (2) This plate was also milky cloudy, but the colonies were larger and not nearly so numerous, about 95 per cent having been destroyed by the heat.

These two plates were kept under observation for a week or two, but with no conflicting results.

IX. June 3, 1897, six tubes of stock 245, a beef broth made feebly alkaline to litmus by means of sodium carbonate, were selected for this experiment. Each was inoculated with a loop from tube 4, June 2, a 26-hour culture in stock 245, which was not yet distinctly clouded, but became so after a few hours. Four tubes were heated, but not until over an hour after inoculation (room temperature 28° C.) The temperature of the bath was unusually variable, ranging from 46.70° to 47.10° C., it being most of the time below 47° C.

Results: (1) Two of the tubes were cooled slowly at room temperature. These tubes were examined at intervals of a few days until July 29, but both remained clear.

(2) Two of the tubes were cooled quickly under running water. One of these tubes remained clear for 56 days, after which the experiment was discontinued. The other remained clear until the sixth day. It then became feebly clouded, and contained numerous small zooglœæ, most of the germs seeming inclined to pass at once into this state, i. e., growth was retarded but not all of the germs were killed. June 10, feebly clouded, zooglœæ larger, numerous, ragged. June 14, moderately clouded; slight rim on tube at level of liquid; the larger zooglœæ are distinctly yellow. June 16, well clouded with rolling clouds on shaking. Considerable distinctly yellow precipitate. A thin pellicle in shape of a delicate membrane thickly dotted with small zooglœæ is present. This membrane sinks on gentle shaking, breaking up into ribbons which are fine granular under ×6 Zeiss aplanat. June 28, copious yellow precipitate. The pseudo-pellicles have all settled. July 6, abundant yellow precipitate. Fluid nearly clear. On this date the other three (sterile) tubes were reinoculated from this tube, but they remained clear.

(3) Two of the tubes were kept as checks. One of them became contaminated with a white organism growing best on the bottom of the tube (Oospora?). The other remained clear until after the third day. On the fifth day it was distinctly but feebly clouded, and the surface layers contained small zooglœæ which streamed down cloudily on gentle shaking. June 9, clouded more than yesterday, but not heavily so. June 10, well clouded with considerable yellow precipitate. June 14, a pellicle consisting of yellowish more or less united zooglœæ. June 16, well clouded with rolling clouds on shaking. No new pellicle. The broken one (shaken down on the 14th) has not gone to pieces, but lies on the bottom with hundreds of tiny zooglœæ embedded in it very regularly. June 28, a copious yellow precipitate. July 6, fluid nearly clear, i. e., becoming exhausted of nourishment; otherwise as before. July 29, washed out; precipitate yellow.

X. On June 3 three tubes of stock 244b (+20 gelatin) were converted into 3 poured plates as follows:

(1) One cubic centimeter of the cloudy fluid from tube 4, June 2 (see IX), was

transferred to 10 c. c. of gelatin at 30° C., shaken, and poured into a sterile Petri dish to form the check plate.

(2) The remainder of the culture (approximately 9 c. c.) was then plunged for 10 minutes into water at 46.50° to 46.60° C., and another 1 c. c. immediately pipetted out into another tube of 10 c. c. of gelatin at 30° C., shaken, and poured into a second Petri dish.

(3) The culture was then allowed to cool to room temperatures after which 1 c. c. was pipetted into another 10 c. c. of gelatin at 30° C., shaken and poured into a third Petri dish.

These 3 dishes were then put into the cool box, where they were kept at 12° to 16° C., and examined and compared from time to time in the same way as the dishes of agar.

Results: (1) Colonies to the number of 2,000 to 3,000 per field (Zeiss 16 mm. and 12 comp. ocular) appeared in this dish. (2) More than 80 per cent of the germs were destroyed by the heat, i. e., there were only 200 to 600 colonies per field in this dish. (3) About 400 colonies per field appeared in this plate.

The culture from which these 3 plates were inoculated was made with a single loop from a broth culture 11 days old and this fact, together with its own age (28 hours at 24° to 28° C.), precludes the idea that spores played any part in the results obtained.

XI. February 3, 1898: Six tubes of alkaline beef broth, stock 286b (stock 286 consisted of the broth from 1,000 grams of minced lean beef covered with 1,500 c. c. distilled water and left in the ice box 24 hours. The fluid was finally made up to 2,000 c. c., titrated, and divided into four equal parts. Stock 286b received enough $\frac{2N}{1}$ NaOH to render it exactly neutral to phenolphthalein, i. e., strongly alkaline to neutral litmus paper). These tubes were of Weber's resistant glass, 169 by 17 mm., and very thin walled. Each received exactly 10 c. c. of the broth, in which, from previous tests, the germ was known to grow readily, even when added in very small quantities. Each of these 6 tubes was inoculated with a drop of fluid from tube 1, Jan. 29, a beef broth which was nicely clouded with good rolling clouds on shaking. This broth had been clouded about 55 hours, but showed as yet very little precipitate and no pellicle or zoogloeæ. As much fluid was put into each tube as could be lifted out on a medium sized (2 mm.) loop and 5 or 6 cm. of wire above it, i. e., an enormous number of germs, as microscopic examination showed. About 15 or 20 minutes after inoculation 4 of the tubes were plunged into the hot water, while the other 2 were held as checks. The exposed tubes were put well down into the bath so that the surface of the broth was 5 to 8 cm. below the surface of the water, which was in constant motion. The exposure was exactly 10 minutes. On removal, 2 of the tubes were cooled immediately under flowing water, while the other 2 were allowed to cool gradually at room temperature (23° C.). All were then screened from the diffused light of the room and set away at room temperatures which varied from 15° to 25° C. The temperature of the water bath at the beginning was 47.80° C., falling slowly to 47.58° C. at the end. During the middle 8 minutes the range of temperature was from 47.70° C. to 47.60° C. These tubes were under observation 33 days.

Results: (1) Checks. Both tubes clouded inside of 48 hours and passed through a normal course of growth. (2) Cooled quickly. Both tubes remained perfectly clear. (3) Cooled slowly. Both tubes remained perfectly clear.

XII. February 3, 1898: This experiment was in all respects a duplicate of the preceding, except that the water was a trifle cooler and that after inoculation the tubes were allowed to stand one-half hour before plunging. The temperature of the water was 47.45° C. at the beginning and 47.17° C. at the close. After one minute the temperature of the bath fell to 47.30° C. and during the next 5½ minutes it gradually fell to 47.20°. During the remaining 3½ minutes the temperature fluctuated between 47.16° C. and 47.18° C., being at the latter point most of the time.

Results: (1) Checks. Both tubes clouded inside of 48 hours and developed normally. (2) Cooled quickly. Both tubes remained perfectly clear till the end of the experiment (33 days). (3) Cooled slowly. Both tubes remained clear until the ninth day. Then one of them became very feebly clouded and gradually passed through the same changes as the check tubes, but never caught up with the latter. The other tube continued clear till the end of the experiment.

As a result of these experiments we may conclude that exposure of *Ps. hyacinthi* for 10 minutes to a temperature of 43° C. does not appreciably retard growth; 44° retards growth slightly; 45° retards considerably; 46° to 46.50° destroys the greater part of the organisms; 47.17° to 47.45° (mostly 47.20° to 47.30°) destroys almost all; 47.58° to 47.80° (mostly 47.60° to 47.70°) destroys all.

The thermal death point, therefore, under the exact conditions named, may be recorded as approximately 47.50°, but a majority of the rods are killed at 46.50° C.

Probably some of the rods are destroyed by 10 minutes' exposure to temperatures as low as 45° or 45.50°. Exposures for much longer periods to temperatures a few degrees lower, e. g., 7 days at 40° C., have the same effect, as may be seen from what follows.

The thermal death point of *Ps. stewarti* in +15 beef bouillon is approximately 53° C. In Uschinsky's solution it is a little higher.

The thermal death point of *Ps. phaseoli* is approximately 49.50°, and that of *Ps. campestris*, is 51.50°.

MAXIMUM TEMPERATURE FOR GROWTH.

The maximum temperature at which *Ps. hyacinthi* will grow in favorable media is 34° to 35° C., the exact temperature limit varying somewhat with the medium used and with the heat resistant power of individual rods. This conclusion rests on the following experiments, which were made in a Rohrbeck thermostat, covered with thick haircloth and provided with a large water reservoir, so that the culture chamber is not quickly sensitive to changes in gas pressure or in the temperature of the room.

(1) In stock 244c (0 gelatin), kept in the thermostat at 40° C., there was no growth whatever, and none appeared when this tube was removed from the thermostat at the end of 7 days and kept at room temperatures for an additional 38 days. This tube was inoculated with a very large loop from a beef-broth culture, which had been cloudy for 6 days. In a second tube of this gelatin, inoculated from the same culture at the same time and in the same manner, but kept throughout at room temperatures of 24° to 34° C. (mostly 25° to 29°), the organism developed normally, clouding the fluid in 24 hours.

(2) Three potato cylinders (stock 246) were inoculated at the same time and from the same culture as the 2 tubes of gelatin. One of these was put into the thermostat at 40° C. and the other 2 were kept at room temperatures.

Result: In each of the 2 check tubes the organism developed normally, the first distinct sign of yellow growth being visible in about 47 hours. No trace of growth appeared in the tube which was put into the thermostat, although a very large loop of broth was used in making the inoculation, and there was plenty of water in the bottom of the tube. After 7 days this tube was removed from the thermostat and kept in the dark at room temperatures for 38 days, but no growth ensued.

(3) Three well-plugged tubes of 1:2 moderately litmus alkaline beef broth (stock 245) were inoculated at the same time from the same tube, and in the same way. Two of these were put into the thermostat at 40° C. and the other was kept at room temperatures.

Result: The check tube clouded on the fourth day and passed through a normal course of development. The tubes in the thermostat remained perfectly clear until the end of the experiment (45 days).

(4) Three tubes of cauliflower broth (stock 217), which by long standing had dried out one-fifth (2 c.c.), were also inoculated at the same time from the same culture and in the same way. Two of these tubes were kept at room temperatures and the third was put into the thermostat at 40° C.

Result: One of the check tubes clouded on the third day, the other some time between the fourth and seventh day. Both developed a yellow pellicle and threw down a yellow precipitate. The tube in the thermostat was under observation 45 days, but there was no growth.

(5) Three tubes of 1:2 acid beef broth (stock 204) were each inoculated with a large loop from a beef-broth culture of *Ps. hyacinthi* 7 days old. This culture, which was moderately cloudy, showed many small zoogl̄œæ floating in the fluid, and on the bottom a small amount of decidedly yellow precipitate. Two of these tubes were put into the thermostat at 36° to 38° C. and the third was kept at room temperatures (mostly 21°).

Result: On the third day the check tube became feebly clouded and contained many tiny zooglœæ. On the eleventh day this tube was moderately cloudy, showed a yellow precipitate, and bore on the wall of the tube at the surface of the fluid a yellow rim of loosely adhering zooglœæ. An agar culture inoculated from the same tube at the same time and kept at room temperatures also developed normally. The tubes in the thermostat remained free from bacterial growth as long as the experiment continued (22 days).

(6) Three tubes of resistant glass, each containing 10 c. c. of strongly alkaline beef broth (stock 286b, neutral to phenolphthalein), in which *Ps. hyacinthi* was known to grow well, were each inoculated with a loop from a clouded tube of alkaline beef broth 6 days old. After remaining for an hour at room temperatures, 2 of these tubes were put into the thermostat and kept at 35° to 36.35° C. during the first 5 days, then at 32° to 33.50° for 24 hours, and afterwards at 34.15° to 35.35°. The third tube was kept in the dark at room temperatures ranging from 18° to 23° C., except on one day when the room temperature fell to 8° C. Each of the tubes put into the thermostat received a large loop of the cloudy broth; the tube left at room temperatures received a smaller loop of this broth, i. e., not one-fourth as many germs.

Result: In 43 hours the check tube was distinctly clouded. On the fourth day it was well clouded, free from zooglœæ, and showed some yellow precipitate. The other 2 tubes remained clear as long as they were left in the thermostat. One was removed on the thirteenth day and left for 24 days at room temperatures (mostly 22° C.), but no growth ensued. The other was removed on the sixth day and left at room temperatures 31 days, but no growth ensued. At the close of the experiment the tubes still contained 8 c. c. of broth, i. e., the concentration was not beyond what this organism bears readily.

(7) Three cylinders of sugar beet (stock 292) were inoculated at the same time and from the same tube as the preceding, each tube receiving a large loop of the cloudy fluid. Two of these tubes were put into the thermostat along with the beef broth (6), and the third was kept at room temperatures.

Result: The check tube showed no growth at the end of the fourth day, i. e., there was some retardation. On the sixth day, when next examined, there was a distinct yellow growth over a large part of the cylinder. On the eighth day this growth was bright yellow and copious. The development of this culture was normal, and continued for a month or more. The 2 tubes put into the thermostat remained free from bacterial growth. Both were taken out on the thirteenth day and left at room temperatures (19° to 26° C.) for 54 days, but there was never any growth.

(8) Four well-plugged tubes of resistant glass, containing 10 c. c. of strongly alkaline beef broth (stock 286b), which had evaporated to 8 c. c. by long standing, were each inoculated with a large loop from a beef-broth culture of *Ps. hyacinthi* 48 hours old, which had been inoculated copiously from a solid culture and was cloudy from growth. Two of these tubes were kept in the dark at room temperatures of 20° to 25° C. The other 2 were put into the thermostat at 33.35° to 35.58° C. (mostly 34.32° to 34.55°) during the first 8 days, and after that at 32.45° to 35.55°.

Result: The 2 check tubes clouded in 48 hours and developed normally. The other 2 tubes remained clear as long as they were kept in the thermostat—37 days for one and 13 days for the other. The latter was removed on the thirteenth day and kept at room temperatures for 24 days, but no growth ensued. The germs were dead, however, in each tube considerably in advance of the thirteenth day, for 2 tubes of the same beef broth which were inoculated therefrom on the eighth day, using large loops, and left in the dark at room temperatures, remained entirely free from growth as long as the experiment continued (29 days).

(9) Two cylinders cut from a yellow turnip and steamed in the usual amount of water were inoculated at the same time, from the same culture, and in the same copious manner as the preceding. One of these was put into the thermostat and the other was kept at room temperatures.

Result: The check tube showed a distinct yellow growth on the third day. On the fifth day this growth was copious and typical for *Ps. hyacinthi*. The tube in the thermostat showed no growth on the fifth day and was then reinoculated with a large loop of yellow slime from the check tube. The tube was then shaken until the slime was washed over the cylinder and dissolved in the fluid, and the yellow color invisible. The tube was then put back into the thermostat. In 26 hours there was a slight yellow growth on the upper part of the cylinder (temperature 34.45°, falling slowly to 33.35° C.). Two days later there was, apparently, no increase of growth (temperature 34.53° a. m., 34.15° p. m., 34.32° a. m.), and not one one-hundredth part as much growth as in a tube inoculated at the same time for comparison. On the eighth day (temperatures 34.40° to 35.55° C.) growth was very scanty and the color scarcely visible. The amount of growth at this time was not one three-hundredth as much as in the check tube held at room temperatures. On the twelfth day after this reinoculation growth had increased a little, but was still very feeble and certainly not one one-hundred and fiftieth as much as the same culture would have given at room temperatures. During these last 4 days the thermostat was considerably cooler, the temperature of the culture chamber ranging from 32.45° to 34.45° C., and being most of the time below 34°. After 49 days in the thermostat a tube of alkaline beef broth was inoculated very copiously from this tube and left at room temperatures 27 days, but no growth ensued, i. e., the vegetative rods were dead and no spores were present.

(10) Two cylinders of steamed sugar beet were inoculated at the same time, from the same culture, and in the same manner as in the two preceding experiments. One of these tubes was put into the thermostat and the other was held at room temperatures.

Result: On the fifth day there was no visible growth in either tube and both were reinoculated very copiously with the solid slime from a turnip culture 5 days old (the check of series 9). The tube which came from the thermostat was shaken thoroughly before replacing, so that if there were any subsequent growth it might not be confused with any undissolved slime used in making the inoculation. At the end of 26 hours there was a slight growth on the cylinder in each tube. On the third day, in the thermostat (temperatures 34.45° p. m., 34.35° a. m., 33.35° p. m., 34.53° a. m., 34.15° p. m., 34.32° a. m.) the germs covered 2 sq. cm. on one side of the cylinder. This growth was plainly yellow but extremely thin. On the fifth day (temperatures 34.32° p. m., 35° a. m., 34.40° p. m., 34.85° a. m.) there seemed to be a slight increase in growth. This growth was very thin, distinctly yellow, not smooth, and rather dry, i. e., not wet-shining. In the check tube there was from 10 to 20 times as much growth, but not as much growth as there should have been, owing to the fact that the check cylinder was rather dry. On the eighth day (temperatures 34.85° a. m., 34.55° p. m., 35.55° a. m., 35.45° p. m., 34.83° a. m., 34.65° p. m., 34.95° a. m.) there was some increase, the growth being distinctly yellow, but too thin to hide minute irregularities of the substratum. The volume of growth at this time was not one-fiftieth that in the check tube. Examined microscopically, this growth consisted of zooglœæ, short rods and long rods. The short rods were single, in doubles, or in fours; the long rods were slender threads, 10 to 20 or more times the length of an ordinary rod. These threads were numerous and their segments were not well defined. No involution forms were observed or any bodies suggestive of spores. On the twelfth day (temperatures 34.05° a. m., 33.35° p. m., 34.45° a. m., 33.35° p. m., 32.75° a. m., 32.45° p. m., —— a. m.) the growth was meager, thin, dull yellow, and its surface was shagreened. There was no yellow slime in the water, but the germs on the cylinder out of the water appeared as if still growing, although very slowly. After 49 days in the thermostat a large loop of slime from this tube was removed and put into alkaline beef broth. This tube was kept at room temperatures for 27 days, but no growth ensued.

(11) This experiment was undertaken to see if cultures started at room temperatures would not do better when put into the thermostat than those which had been inserted soon after inoculation. For this purpose I selected a tube of alkaline beef broth, which had been kept as a check on series No. 8, and a tube of yellow turnip, kept as a check on series No. 9. The turnip culture was put into the thermostat on the fifth day, at which time there was a copious, yellow, wet-shining, homogeneous-looking growth covering most of that part of the cylinder out of the water. The tube of beef broth was put in on the eighth day, at which time the fluid was moderately cloudy and had thrown down a little yellow precipitate, but had not yet developed any pellicle, rim of germs, or zooglœæ. The temperatures were 34.15° to 35.55° during the first 9 days (once as low as 33.35°) and then 32.45° to 34.45° C. There was no exact check tube for the turnip, but a transfer was made from it into another tube of the same medium; for comparison with the beef broth the other check tube of series No. 8 was used.

Result: (a) The beef broth in the thermostat at once fell behind the check tube in growth. On the fifth day the clouding appeared to be feebler than on the start and the trifling precipitate had increased proportionately to the decrease in clouding, but scarcely more. The check tube was distinctly cloudier. On the ninth day there was no increase of precipitate. On the twenty-ninth day there was no pellicle, no rim of germs, no zooglœæ, and not more precipitate than on the fifth day, i. e., there appeared to have been no growth whatever during the whole time of the exposure. On this date the check tube was uniformly clouded, showed a yellow rim, and had thrown down a yellow precipitate 12 mm. broad and 2 mm. deep. On the twenty-ninth day a large loop of fluid was taken from the tube in the thermostat and put into a sterile tube of the same beef broth. This tube was under observation 17 days, in conditions very well suited for growth, but no growth ensued. At the end of 46 days in the thermostat this experiment was repeated, inoculating copiously into alkaline beef broth diluted with distilled water. The tube was kept at room temperatures and watched for 27 days, but no growth ensued, i. e., no spores were present. (b) On the fifth day in the thermostat the slime on the turnip cylinder was still wet-shining, but it was not as homogeneous looking, being uniformly mottled lighter and darker yellow. On the eighth day the culture was less vigorous and the substratum had browned slightly. The slime was now examined microscopically for several hours. It consisted of the ordinary short rods and of slender threads which were of the same diameter as the rods but were often 50 times as long. These threads were numerous. No involution forms were observed nor any bodies resembling spores. On the twelfth day the culture was in a much worse condition. Growth had ceased and the slime out of the water had so much dried out that the substratum under it was now visible. Nineteen days after this date the turnip cylinder which had been inoculated from this tube and kept at room temperatures was still covered with a thick, smooth, wet-shining, homogeneous-looking, pale yellow layer of slime, entirely hiding the substratum. The fluid in the bottom of the tube was also grown full of the slime, which was not the case with the culture in the thermostat. After 49 days in the thermostat a large loop from this tube was put into alkaline beef broth and watched at room temperatures for 27 days, but no growth ensued.

(12) Two steamed cylinders of carrot (stock 290), standing in several cubic centimeters of distilled water in tubes of resistant glass, were each inoculated with a large loop of the yellow slime of *Ps. hyacinthi* from recent growths in a turnip culture 5 days old. These tubes were then shaken until the slime was dissolved in the water and washed over the cylinder. One of the tubes was put into the thermostat at 33.35° to 35.45° C. (mostly 34.35° to 35°) and the other was held at room temperatures.

Result: On the third day the check tube showed a plentiful yellow growth, covering nearly all of one side of the long cylinder. On the fifth day this growth was dense enough to hide the orange color of the substratum. The tube in the thermostat, on the eighth day, showed no growth whatever, although it still held 2 c. c. of water and was consequently moist. This tube was now removed to room temperatures of 19° to 25° C. On the fourth day thereafter a copious, smooth, wet-shining, homogeneous-looking, bright yellow growth, dense enough to hide the substratum, covered about 3 sq. cm. of the inoculated cylinder.

(13) Two cylinders from a yellow, flat-bottomed turnip, prepared in the same way as the carrot, were inoculated at the same time as the latter and from the same culture, each tube receiving a large loop of the yellow slime. These tubes were then shaken until the slime was dissolved in the water and spread over the cylinder. One of the tubes was held at room temperatures and the other was put into the thermostat. The tube in the thermostat contained several cubic centimeters of water; the check tube contained only a small amount of water.

Result: On the third day, in the check tube, there was a copious, smooth, wet-shining, yellow growth over nearly the entire cylinder. On the fifth day this growth had become more abundant, covering the whole cylinder and filling up the small amount of fluid in the bottom of the tube. The other tube was left in the thermostat 8 days at 33.35° to 35.55° (mostly 34.35° to 35°), during which time no growth was visible either to the naked eye or with a Zeiss × 6 aplanat. The tube was now removed to room temperatures of 20° to 25° C. On the fourth day after this removal two-thirds of the cylinder (all out of the water) was covered with a copious, yellow, smooth, wet-shining, homogeneous-looking bacterial layer, which developed normally for *Ps. hyacinthi*.

(14) Four tubes of 1:2 acid beef broth (stock 286a, acidity + 25), originally holding exactly 10 c. c., but dried out about one-fifth by long standing and consequently more acid than the original stock, were each inoculated with two large loops from an alkaline beef broth culture of *Ps. hyacinthi* 10 days old. This culture was uniformly clouded, and showed considerable yellow precipitate, but there were no zoogl≈ìæ and the rim of germs was only commencing to form, i. e., the fluid was crowded full of living germs and in excellent condition for use. Two of the tubes

inoculated therefrom were set away in the dark at room temperatures of 19° to 25° C. (mostly 21° to 23°). The other two were put into the thermostat at 34.55° to 35.55° for the first 4 days and then at 32.45° to 34.45° C.

Result: The check tubes were feebly clouded on the third day. They were first examined at the end of 72 hours, and probably clouding could not have been detected more than 6 or 8 hours earlier. These two cultures passed through a normal development. The other tubes were left in the thermostat 27 days, during all of which time they remained perfectly clear. On the twenty-seventh day both were removed to room temperatures and watched for 6 weeks, but they never clouded. When removed from the thermostat each tube still contained about 6.5 c. c. of fluid.

The following inferences respecting *Ps. hyacinthi* appear to be warranted by these experiments:

(a) The organism will not grow on any medium at 40° C., and after 7 days exposure to this temperature it will not grow at any temperature. Probably a much shorter exposure to 40° C. would kill it.

(b) The organism will not grow in unneutralized (acid) beef broth at 36° to 38° C., and consequently it is not likely that it will prove pathogenic to warm-blooded animals.

(c) The organism will not grow in strongly alkaline beef broth at 35° to 36.35° C., and after 6 days' exposure to this temperature it will not grow at any temperature.

(d) The organism will not grow on sugar-beet cylinders at 35° to 36.35° C., and after 13 days' exposure to this temperature will not develop at any temperature.

(e) The organism will not grow in strongly alkaline beef broth at 34.15° to 35.58° C., and after 8 days' exposure to this temperature it will not grow at any temperature.

(f) When inoculated very copiously from a young solid culture, the organism grew scantily on yellow turnip at 33.35° to 34.45° C.

(g) When inoculated very copiously from a young solid culture, the organism grew very feebly on sugar beet at 34.15° to 35° C.

(h) Growth already well under way in strongly alkaline beef broth and on yellow turnip was stopped at 34.15° to 35.55° C.

(i) In 8 days the organism made no visible growth on steamed carrot at 33.35° to 35.45° C., but all of the germs were not killed.

(k) In 8 days the organism made no visible growth on yellow turnip at 33.35° to 35.55° C., but all of the germs were not killed.

(l) In 27 days the organism made no growth in unneutralized (acid) beef broth at 34.55° to 35.55° C., and all were dead before the twenty-seventh day.

Ps. stewarti refused to grow at 40° C., in Uschinsky's solution and in strongly alkaline beef broth (0 of Fuller's scale). It grows in the thermostat at 36° to 37° C., on most media, but not so well as at room temperatures of 24° to 25° C. *Ps. campestris* did not grow at 40° C., and grew not at all or very feebly at 37° to 38° C.—i. e., about as *Ps. hyacinthi* grows at 34° to 35° C.

Optimum Temperature for Growth.

No special experiments have been instituted to determine at what temperature growth of *Ps. hyacinthi* is most rapid, but from a careful collation of the records of several hundred cultures made during the past four years and kept at room temperatures—i. e., of all cultures which were examined frequently enough during the first few days of growth, and for which the necessary temperature records were set down—I find that, on good media, growth was slow at 10° to 12° C., moderate at 18° to 25° C., and fast (for this organism) at 28° to 30° C. These cultures were instituted at all seasons of the year, and sometimes for several days together the room temperature would be nearly stationary—e. g., at 18°, 25°, or 30° C. In a few instances I have thus been able to compare at different temperatures the rate of growth when the inoculations were made with the same amount of material taken from cultures of the same age and kind

Using these records, therefore, as a basis for judgment, the optimum temperature for growth may be placed at 28° to 30° C.

Minimum Temperature for Growth.

On very favorable media this is believed to be about 4° C. for *Ps. hyacinthi*. Only four sets of experiments have been made. (1) On a sugar beet cylinder inoculated copiously with bright yellow slime from a starch jelly culture 8 days old and kept in the ice chest at 10° to 12° C. (temperature possibly at times as low as 7° or 8° C., but never lower) no visible growth appeared in 12 days. The tube was now removed to room temperatures. Five days afterwards there was a distinct yellow growth covering more than 2 square centimeters of the surface.

(2) A tube of unneutralized 1:2 beef broth (stock 204), inoculated with a large loop from a well-clouded beef broth culture 7 days old and put into the ice chest at 10° to 12° C., was clouded very feebly at the close of the fifth day. A check tube at 21° C. clouded feebly in 67 hours.

(3) Two freshly prepared cylinders of coconut, standing in test tubes in an abundance of distilled water, were each inoculated with approximately 1 c. mm. of yellow slime from a coconut culture 4 days old. These tubes were put into the ice chest. In 42 hours there was a slight but distinct growth in each tube, the temperature, however, had been higher than was anticipated—i. e., 10° to 15° C. These tubes were now shaken for 10 minutes—i. e., until all trace of the yellow growth was washed off and dissolved in the fluid. They were then put back into the chest with a larger quantity of ice. On June 2, 4 p. m. (after 54 hours), there was a slight growth in each tube, although the temperature had remained under 8° C. On June 3, 9 a. m. (tem-

perature 8.2° C.), there had been some further growth. On June 4, 9 a. m. (temperature 8.5° C.), there was a distinct increase of growth over what was present 24 hours earlier. One of the two tubes was now removed to room temperatures of 25° to 26° C. During the next 26 hours the growth in this tube doubled. During the same period there was a slight growth in the other tube (temperature 8.5° C.). At this time, in this tube, a bright yellow growth covered more than 1 sq. cm. of the surface where 5 days before (after the shaking) no growth whatever was visible. All of this growth took place between 7.4° and 9° C., the temperature most of the time during the 5 days ranging between 7.5° and 8.5° C.

(4) Four tubes of strongly alkaline beef broth (stock 382 neutral to phenolphthalein) were each inoculated with a 3 mm. loop from a well-clouded beef broth culture 3 days old. One of these tubes was held at room temperatures of 20° to 25° C. This culture was moderately clouded on the third day and passed through a normal growth. The other 3 tubes were placed in the ice chest for 18 days at 2.8° to 4.5° C. (mostly 3° to 4° C.), during the whole of which time they remained perfectly clear. On then removing them to room temperatures they clouded in 16 hours at 21°–23° C. The rapidity with which they clouded when removed from the ice box suggests that the bacteria grew slightly at times while exposed to the low temperature.

The minimum temperature of *Ps. campestris* is not known. It lies below 7° C. The minimum temperature of *Ps. stewarti* is not known exactly, but it is believed to be a degree or two higher than that of *Ps. hyacinthi* for the following reason: Tubes of *Ps. stewarti* were exposed in the ice box at 2.8° to 4.5° C., along with those of the hyacinth germ. There was no clouding in 18 days, and on removing to room temperatures the tubes were not clouded until the third or fourth day, and then only feebly. The check tubes clouded on the second and third days. The fluids used were Uschinsky's solution and an alkaline beef broth (stock 382). Each tube was inoculated with one 3-millimeter loop from a young fluid culture (3 days old). The date of clouding on removal indicates clearly that, contrary to the case of *Ps. hyacinthi*, there had been no growth whatever during the 18 days' sojourn of the tubes in the ice chest.

FORMATION OF ACIDS.

With exception of the production of a small amount of acid from ethyl alcohol (probably acetic acid), the formation of acids by *Ps. hyacinthi* is rather obscure, in spite of all the attention I have given to it. At times, especially when small quantities of the carbohydrate were used, no acid was detected from the growth of this organism in the presence of sugars. Even when large quantities of the various sugars were used there was no prompt change from alkaline or neutral to acid. After some weeks, however, many of these cultures changed from alkaline to neutral, and others became decidedly acid, and the acidity increased on concentration by boiling rather than diminished. It would seem, therefore, that a small quantity of some non-volatile acid is formed by this organism from a variety of substrata, but that the formation of this acid is in no way associated with facultative anaerobism or with the production of gas.

The other yellow organisms, so far as tested, behaved in the same way as *Ps. hyacinthi*, so far as relates to the slow development of a non-volatile acid in the presence of certain sugars and of certain vegetable substances rich in sugars.

FORMATION OF ALKALIES.

Feebly acid or neutral culture media of various kinds were finally rendered alkaline by *Ps. hyacinthi*, but not rapidly so, and all the tests instituted lead me to the conclusion that this organism is a relatively feeble alkali producer. This alkali is volatile, and a part of it, at least, is undoubtedly ammonia. Neutral or acid reactions were observed in the following old and very old cultures: Carrot, sugar beet, sweet potato, yellow globe turnip, grape sugar agar, cane sugar agar, nutrient starch jelly with cane sugar, nutrient starch jelly with glycerin. The following culture media became and remained alkaline: Potato, cocoanut, ordinary nutrient agar, salted peptone water, milk, milk with grape sugar, milk with methyl alcohol, milk with glycerin, hyacinth broth.

The results obtained by special tests are given under the following heads:

Rosolic Acid Test.

The action of *Ps. hyacinthi* on rosolic acid was tested in Dunham's solution. To each 100 c. c. of this salted peptone water was added 1 c. c. of a solution made of 0.5 gram rosolic acid; 20 c. c. distilled water; 80 c. c. absolute alcohol. The alkali in the peptone (Witte's) made this culture medium too red, and the fault was remedied by adding to each 90 c. c. of the solution 6 drops of $\frac{2N}{1}$ HCl, which rendered the medium yellowish and suitable for the experiment. The results obtained with this organism and with others used for comparison are given in the following table:

TABLE VI.—*Behavior of Ps. hyacinthi and other bacteria in salted peptone water with rosolic acid and HCl (stock 493). Original color of fluid pale orange yellow.*

Organism.	Growth.	Change in color.			
		First week.	Second week.	Third week.	Fourth week and later.
Ps. hyacinthi	Moderate after a time, but none visible on 3d day, and feeble on 6th.	3d day, no change in color; 6th, no reddening.	9th day, paler than check; 11th, color gone.	16th day, all the yellow color has gone and no red has come; fluid colorless but clouded: 21st, colorless.	28th day, fluid colorless or faint rose color when looked through endwise; precipitate rosy or salmon; 37th, no red color—colorless in comparison with check; 56th, no red color.
Ps. campestris	Good; feebly clouded on 3d day; not heavily clouded on 9th.	3d day, no change in color; 6th, no reddening.	9th day, a distinct change to pink; this was first observed on 8th.	16th day, fluid geranium red, the only culture showing any distinct red; 21st, as on the 16th.	28th day, fluid deep red, approximately Ridgway's scarlet; 37th, as on 28th, but color deeper, i. e., poppy red; 56th, the deep red color persists.
Ps. stewarti............	Good; well clouded on 3d day.	3d day, no change in color; 6th, no reddening.	9th day, no reddening; 11th, no reddening.	16th day, color not deeper than check, but rosaceous rather than yellowish; 21st, slight, if any, change since 16th.	28th day, a decided change; the fluid is now plainly pale red, i. e., R.'s flesh color, with a trifle of pink in it; color about one-fourth as deep as in tube of Ps. campestris; 37th, much more color; it is now geranium red. This medium alone would serve to separate Ps. stewarti from Ps. hyacinthi, if the results here obtained prove constant; 56th, the color now lies between scarlet vermilion and geranium red.
B. pyoc. pericarditidis .	Good; well clouded on 3d and 9th days.	3d day, no change in color; 6th, no reddening.	9th day, no reddening; like check in color; 11th, no reddening.	16th day, like Ps. stewarti, colonies on the rim are yellowish-salmon; 21st, no distinct change.	28th day, red color developing, about one-half as deep as tube of Ps. stewarti, i. e., R.'s pale flesh color; 37th, the color has deepened; the fluid is now deep flesh color, i. e., a deeper color than R.'s No. 18; 56th, color pale geranium red, i. e., much deeper than it was.
B. coli (a strong indol producer from Dr. Theobald Smith).	Good; well clouded on 3d and 9th days.	3d day, no change in color; 6th, no reddening.	9th day, resembles check in color; 11th, no reddening.	16th day, like Ps. stewarti and B. pyoc. pericard.; 21st, color not deeper than check, but slightly rosaceous, in marked contrast to Ps. campestris.	28th day, fluid a pale red, same depth and same shade as in tube of B. pyoc. pericard.; 37th, fluid now pale flesh color; 56th, color slowly deepening; it is now midway between flesh color and geranium pink.

TABLE VI.—*Behavior of Ps. hyacinthi and other bacteria in salted peptone water with rosolic acid and HCl (stock 493). Original color of fluid pale orange yellow.*—Continued.

Organism.	Growth.	Change in color.			
		First week.	Second week.	Third week.	Fourth week and later.
B. amylovorus.........	Good; well clouded on 3d and 9th days; moderately cloudy on 16th.	3d day, no change in color; 6th, no reddening.	9th day, resembles check in color; 11th, no reddening.	16th day, color same as in tube of Ps. stewarti; 21st, color not deeper than in check tube, but feebly rosaceous instead of yellow.	28th day, as on 21st; 37th, distinctly paler than B. coli, same depth of color as check, and nearly the same orange yellow, but with a trifle of rose in it; 56th, a decided change in color; the fluid is now midway between flesh color and geranium pink.
B. carotovorus	Good; well clouded on 3d, 9th, 16th, and 28th days.	3d day, no change in color; 6th, no reddening.	9th day, no reddening; if any change, paler than check.	16th day, color gone; fluid as colorless as in tube of Ps. hyacinthi; 21st, as on 16th.	28th day, no color except in the precipitate, which is salmon. This organism and Ps. hyacinthi are alike in having destroyed the original yellowish color of the fluid without the production of any red color, and in having a stained precipitate; 37th, fluid colorless, precipitate orange brown; 56th, fluid colorless.

ACID FUCHSIN TEST.

The action of *Ps. hyacinthi* on acid fuchsin was tested in peptone water. The culture medium was prepared as follows:

200 c. c. distilled water.
2 gm. Witte's peptonum siccum.
4 c. c. acid fuchsin water.
10 drops $\frac{2N}{1}$ HCl (to counteract the alkalinity of the peptone).

The acid fuchsin water consisted of 150 mg. of Grübler's Fuchsin S. (after Weigert) dissolved in 30 c. c. of distilled water.

The tubes each contained 10 c. c. of the rose-red fluid. They were inoculated on March 21. Tubes 1, 2, and 3 were inoculated from fluid cultures; tubes 1′, 2′, and 3′ were inoculated from solid cultures. The results obtained with *Ps. hyacinthi* (tubes 1 and 1′), *Ps. campestris* (tubes 2 and 2′), *Ps. stewarti* (tubes 3 and 3′), *B. pyoc. pericarditidis* (tube 4), *B. coli* (tube 5), *B. amylovorus* (tube 6), and *B. carotovorus* (tubes 7 to 10) are summarized below:

March 24.—Slight variations in color, but each tube paler than the check tubes.

March 27.—Nos. 1′, 2, 2′, 3′, 4, and 5 are much alike in color. They have faded considerably; i. e., they are now rose color. Nos. 1, 3, and 6 are deeper red. None are colorless, but all except 7–10 are paler than on the twenty-fourth.

March 30.—There has been a marked loss of color in 1, 1′, 2, 2′, 4, and 5, and the fluids in these tubes are now only pale pink. In 3, 3′ and 6 there has been only a moderate fading.

April 6.—About one-tenth of the color is left in 1 and 1′; i. e., 1 c. c. of the red fluid from a check tube diluted with 9 c. c. of water gives a color a trifle deeper than that in these tubes. Only one-twelfth to one-fifteenth of the color remains in 2 and 2′. In 3 about one-fifth of the color remains, in 3′ about one-eighth, in 4 about one-tenth, in 5 about one-ninth, in 6 about one-seventh. In 7–10 there is no fading.

April 11.—The cultures still fall into three groups, i. e.: (a) Those in which nearly all of the color has disappeared, viz, *Ps. hyacinthi*, *Ps. campestris*, and *B. pyoc. pericarditidis*. (b) Those in which a considerable portion of the color remains, viz, *Ps. stewarti*, *B. coli*, and *B. amylovorus*. (c) Those in which the color remains the same deep red as on the start, viz, *Bacillus carotovorus*.

April 18.—About one-twentieth of the color is left in 1 and 1′; precipitate yellow. Only about one-fortieth of the color remains in 2 and 2′. In 3 there is about 5 times as much color as in 1 and 1′; in 3′ about twice as much. The color in the latter tube is Ridgway's rose pink. The precipitate in 3 and 3′ is yellow; it is most abundant in 3′. No. 4 is like 1 and 1′; precipitate white. In 5 and 6 the color is rose pink; precipitate white, more copious in 5 than in 6. In 7–10 a slight white precipitate and no change in color.

April 29.—Color gone in 1 and 1′. On looking through the fluid endwise there is a trace of vinaceous buff, but held up vertically to the light (16 mm. diameter) it appears colorless. Nos. 2, 2′, and 4 are like 1 and 1′, and there is no change in 7–10, i. e., it is as red as on the start. The rest of the tubes (3, 3′, 5, and 6) still show some color.

May 16.—Color has not entirely disappeared from 3, 3′, 5, and 6. The color in the 4 tubes of *B. carotovorus* is now only one-half as deep as it was on April 29. The rest are still colorless.

LITMUS.

For tests made with litmus see under Reduction experiments and in various other parts of this paper.

REDUCTION EXPERIMENTS.

METHYLENE BLUE.

The reducing tendencies of *Ps. hyacinthi* and other organisms were tested on methylene blue in Dunham's solution (1 per cent peptone and 0.5 per cent sodium chloride in distilled water). To each 100 c. c. of the Dunham's solution, which was made from Witte's peptonum siccum, was added 2 c. c. of a solution of 50 mg. of methylene blue in 50 c. c. of distilled water. The results obtained are expressed briefly in the following table, each organism grew in the medium, but as there was no repetition of the experiment some of the statements may be subject to revision:

TABLE VII.—*Effect of Ps. hyacinthi, etc., on methylene blue in salted peptone water. Experiment begun March 21. Color of fluid, bright blue.*

Species.	Reduction.	Effect of shaking.	Color at close of experiment.	Precipitate.	Duration of experiment.
Ps. hyacinthi (2 tubes).	Distinct (within a few days), and long continued. Mar. 27, about one-third and one-half as blue as checks. Apr. 11, one-thirtieth and one-fiftieth as much color as in checks. Apr. 18, still nearly reduced. Ps. hyacinthi has a marked effect on methylene blue in this solution, and for a long time. Apr. 25, color begins to return. May 2, as much, and nearly as much, color as in checks.	Color returns quickly and is blue.	Bright blue. Does not deepen on shaking.	Not stained	56 days.
Ps. campestris.....	Distinct (within few days). Mar. 30, one-half as blue as check. Apr. 11, one-fiftieth as blue as check.	Color deepens rapidly on shaking.	Green. Does not change on shaking.	.....do.....	Do.
Ps. stewarti	None. (Observations on Mar. 24, 27, 30; Apr. 6, 11, 18, 25; May 2, 16.) Feebly but distinctly clouded in March, then clear.	No change ...	Bright blue. Same color throughout progress of experiment as on start.	Deep blue .	Do.
B. pyocyaneus pericarditidis (1 tube).	Distinct (on Mar. 30). Apr. 6. Fluid uniformly pale greenish instead of bright blue; distinctly unlike Ps. hyacinthi and Ps. campestris; they are paler than this tube but rapidly deepen their color on shaking, whereas this does not change much even on prolonged shaking. Apr. 11, about one-fifth the color remains. Apr. 25, one-sixth as much color as in check tubes.	Does not change much even on long shaking. Fluid uniformly pale greenish.	Green. Does not change on shaking.	Not stained	Do.

Table VII.—*Effect of Ps. hyacinthi, etc., on methylene blue in salted peptone water. Experiment begun March 21. Color of fluid, bright blue*—Continued.

Species.	Reduction.	Effect of shaking.	Color at close of experiment.	Precipitate.	Duration of experiment.
B. coli (1 tube)	Doubtful. Soon heavily clouded. Paler blue at first (in March), then doubtful. No reduction. Possibly the paler blue on start was due to the heavy clouding.	Does not change. Uniformly blue.	Blue. Does not change on shaking.	Dark blue .	Do.
B. amylovorus (1 tube).	Doubtful. No distinct reduction. Less clouding than in B. coli.	Does not change.	Blue. Does not change on shaking.	Deep blue.	Do.

From the above table the 6 organisms mentioned appear to fall into 4 categories:

(1) Marked reduction, prompt reoxidation on shaking, final color the same as at the beginning—i. e., pure blue. Precipitate not stained. *Ps. hyacinthi.*

(2) As in 1, but the final color of the fluid green. *Ps. campestris.*

(3) Distinct slow destruction of color. Color does not return on shaking. Final color green. Precipitate unstained. *B. pyocyaneus pericarditidis.*

(4) Reduction feeble or doubtful or absent. Final color of the fluid blue. Bacterial precipitate stained deep blue. *Ps. stewarti*, *B. coli*, *B. amylovorus.*

Indigo Carmine.

The reducing tendencies of *Ps. hyacinthi* and other organisms on indigo carmine were tested in the same way as in case of methylene blue. The culture medium consisted of 100 c. c. of Dunham's solution, to which was added 2 c. c. of a solution of 500 milligrams of indigo carmine in 100 c. c. of distilled water. The results obtained are shown in the following table:

TABLE VIII.—*Effect of Ps. hyacinthi, etc., on indigo carmine in salted peptone water; tubes inoculated March 21; color blue.*

Organism.	Mar. 24.	Mar. 27.	Mar. 30.	Apr. 6.	Apr. 11.	Apr. 15.	Apr. 18.	Apr. 27.	May 16.
Check tube (uninoculated).			Color, pale Nile blue.	Greenish color has faded since last record.	Color gone			Colorless	
Ps. hyacinthi (2 tubes).	No distinct change in color.	Fluid a little bluer than in the check tube.	No reduction; fluid deeper blue than check tube.	Color, bright blue, i. e., in marked contrast to check tube.	Color, bright blue; precipitate yellow, 5 mm. broad.	Color, bright blue, but paler, i. e., fading.	Color slowly changing; it is now malachite green.	Color, pale chromium green, i. e., fading.	Color gone, except a pale dirty yellowish hue.
Ps. campestris...	.....do........	.....do........	.....do........	Not now pure blue, but a greenish blue, approximating Nile blue.	Color, paler than it was, i. e., a very pale green.	Color gone ...	Color gone	Color gone	Color gone.
Ps. stewarti......	.....do........	.....do........	.....do........	Not now blue; pale as check, i. e., a greenish color, decidedly paler than Nile blue.	Color gone		Color gone	Color gone	Color gone.
B. pyocyaneus pericarditidis.	.....do........	.....do........	.....do........	Color still a bright blue; this tube showed the deepest blue on Mar. 30, i. e., pure blue.	Color, bright blue; precipitate 5 mm. wide.	Color, bright blue, but paler, i. e., fading.	Color, blue green; Ridgway's glaucous green.	Color nearly malachite green; deeper than tubes of Ps. hyacinthi.	A slight greenish hue still persists, i. e., an olive buff.
B. coli	.....do........	.....do........	.....do........	Color not now blue, but a green as pale as the check.	Color gone		Color gone	Color gone	Color gone.
B. amylovorus...	.....do........	.....do........	.....do........	Not now blue; color a greenish blue, nearly as pale as check.	Color gone, i. e., no greenish blue remains.		Color gone	Color gone	Color gone.

As shown in this table, the development of a deeper, purer blue in all of the cultures, and the persistence of this color in *Ps. hyacinthi* and *B. pyocyaneus-pericarditidis* until long after it had disappeared from the check tube and from the other cultures, were the most interesting results obtained.

LITMUS.

The action of *Ps. hyacinthi* on litmus (reduction, etc.) is shown in the following table:

TABLE IX.—*Behavior of Ps. hyacinthi in litmus broths, and especially in litmus milk with and without alcohols and sugars.*

No. of tubes.	Stock.	Original color.	Growth.	Color first week.	Color second week.	Color third week and later.	Separation of casein begins.	Reduction begins.	Remarks.
1	242, litmus cauliflower broth.			5th day, no change.	9th day, no change in color.	27th day, no reddening on this date or earlier.		14th day, begun; 18th, wholly reduced in bottom and paler in middle; 27th, complete.	No acid.
1	243, litmus potato broth.		Good			18th day, no reddening of the litmus.		18th day, becoming reduced in bottom.	Do.
1	373, milk with alcohol litmus.	Deep lavender; deep as mauve, but not same color; twice as deep as Ridgway's lavender.		No record	10th day, slightly reddish in rim; 12th, more distinct reddish color in rim.	18th day, fluid retains its lavender color, but the rim has become rose color; 22d, fluid lavender, rim and pellicle reddish; 38th, rim and whole body of fluid pale reddish; 48th, curd pinkish lavender.	After 22d day and before 38th day.	? Not before 22d day; whey reduced on 38th.	Feebly acidified.
1	374, milk with alcohol litmus (twice as much as in 373).	A deep violet blue.	Good	do	10th day, rim bluer than in 2 checks; no other change; 13th, rim deeper blue, i. e., more indigo; no red.	16th day, as on 13th; 18th, rim begins to redden, body of fluid violet blue, a trifle bluer than checks; 22d, fluid deeper blue than checks, rim decidedly redder than it was, i. e., burnt carmine; 38th, rim red, fluid wine purple.	No separation (48 days).	No reduction	Do.

TABLE IX.—*Behavior of Ps. hyacinthi in litmus broths, and especially in litmus milk with and without alcohols and sugars*—Continued.

No. of tubes.	Stock.	Original color.	Growth.	Color first week.	Color second week.	Color third week and later.	Separation of casein begins.	Reduction begins.	Remarks.
1	393, milk with alcohol litmus.	Between mauve and aniline violet.	Good	No record	13th day, fluid redder, i. e., between lilac and rose purple; acid reaction appeared some days ago.	17th day, color near rose purple; 27th, rose purple; 50th, rose purple; 78th, rose purple.	No separation (78 days).	Slight on 13th day; none on 27th and 50th.	Germs alive on 50th day.
2	393, milk with alcohol litmus.	....do	....do	....do	....do	17th day, color near rose purple, 27th, rose purple; 50th, rose purple.	No separation (50 days); on slight simmering casein thrown down as an abundant flocculent precipitate.	....do	Germs alive on 50th day; fewer alive in these two and the preceding than on 25th day; on boiling acid vapor and an agreeable smell suggestive of acetic acid; fluid acid after boiling for an hour.
3	394, milk with c. p. litmus.	Very dull hyacinth blue.	....do	....do	13th day, deeper blue than check.	17th day, deeper blue than check, no red reaction; 27th, deep blue, between hyacinth and cyanine; 50th, deep dark blue.	? The litmus itself precipitated some casein on steaming.	No reduction	No acid formed.
1	399, milk with c. p. litmus.	Deep hyacinth blue.	....do	....do	10th day, like checks; 14th, deeper blue than checks.	24th day, deeper blue than checks; 31st, deep blue; 48th, deep blue.	?	....do	No acid.
1	399, milk with c. p. litmus.	....do	....do	....do	10th day, like checks; 14th, deeper blue	24th day, deeper blue than checks no visible change in last 9 days; 48th, rim and	?	None on 24th or earlier; wholly reduced on	No acid formed from the wood alco-

					than checks; on 15th added 2 drops methyl alcohol.	top of fluid deep blue, remainder still reduced.		31st except bright blue patches on wall above fluid.	hol.
2	400, milk with c. p. litmus (more than in 399).	Deep blue	....do	....do	10th day, no change in color; 14th, deeper blue than checks.	24th day, deeper blue than checks; 31st, deep blue; 48th, deep blue.	?	No reduction	No acid.
1	430, milk with c. p. litmus.	Between Ridgway's lavender and royal purple.	Good; distinctly yellow pellicle from 4th day on.	3d day, no change; 6th, slight deepening of blue.	13th day, bluer than checks.	15th day, 13 mm. of loose sediment (flax-flower blue) has settled out of fluid; the latter is dull hyacinth blue; 50th, casein dull hyacinth blue, whey dark purplish; 65th, casein dark hyacinth purple, whey not red by reflected light.	After 9th day; on 13th 6 mm. bluish fluid over the blue casein; on shaking, the whole mass is fluid and uniformly blue and there is no immediate reseparation of whey (15 minutes).	None to 15th day; partial on 22d; the top 3 mm. casein gray, rest pale blue; 27th, only trace of color in whey (at top); casein very pale blue; 43d, wholly reduced.	No acid.
1	430 (litmus milk) with 2 drops methyl alcohol.	Between lavender and royal purple.	Good; 9th day, copious yellow surface growth.	3d day, no change; 6th, bluer than checks; 7th, bluer than checks.	9th day, a little deeper blue than checks; 13th, deeper blue than checks.	15th day, no reddening; 27th, casein dirty gray, whey dull hyacinth blue; 50th, casein deep hyacinth blue, whey dark purple; 65th, casein dull hyacinth blue, whey not red by reflected light.	After 9th day and before 13th.	None to 9th day; slight on 13th. Marked on 22d—i.e., more than in the preceding. Deeper part of casein still blue, upper part gray.	No acid.
1	430 with 4 drops methyl alcohol.	....do	Good	2d day, no change; 5th, bluer than check.	8th day, deep blue—bluer than check, 10th and 11th, bluer than check, 12th, uniformly blue.	15th day, casein lavender blue, 12 mm. deep in a fine precipitate, whey hyacinth blue; 24th, whey dark hyacinth purple; 30th and 45th, casein brownish (still partly reduced); whey deep hyacinth blue.	10th day	24th day, casein dirty gray, 11 mm. deep.	No acid.

TABLE IX.—*Behavior of Ps. hyacinthi in litmus broths, and especially in litmus milk with and without alcohols and sugars*—Continued.

No. of tubes.	Stock.	Original color.	Growth.	Color first week.	Color second week.	Color third week and later.	Separation of casein begins.	Reduction begins.	Remarks.
1	430 with 2 drops ethyl alcohol (absolute).	Between lavender and royal purple.	Good	2d day, no change; 3d, no change; 5th, blue with some purple in it.	Reduced	24th day, color begins to return; the whey is pale burnt carmine; the casein is a dirty yellow gray; 45th, heated 10 minutes at 56° C.; 56th, casein purplish red, whey slightly red by reflected light.	5th day (probably on 4th).	7th day, partial; 8th, all reduced; 12th, all reduced; 30th, all reduced; 45th, all reduced.	Slightly acid.
1	430 with 4 drops ethyl alcohol (absolute).	.....do	Good; a yellow pellicle on 4th day.	4th day, fluid bluer than check; 5th, uniformly blue.	8th day, deep blue—bluer than check. No red color.	17th day, 24 mm. of deep lavender blue casein, 13 mm. dark colored whey; a wide yellow rim and copious yellow precipitate; 23d, whey becoming slowly red, even by reflected light (dull red); 38th, casein purplish gray, whey reddish; 49th, casein purplish red, whey pale red by reflected light.	8th day (1 mm. whey).	23d day, casein now gray.	Slightly acid.
2 tubes	441 (430+2½% grape sugar).	.....do	Good	1st, 2d, 3d days, no change; 4th, no reddening; 6th, no change in one tube; slight deepening of blue in other; 7th as on 6th.	9th day, color same as 2 checks; 13th, still uniformly blue and now deeper than the checks.	27th day, whey dull purple, casein pale lavender blue in one tube and deeper blue in the other; 50th, litmus now slowly oxidizing back; the whey is dark purple; upper part of casein dull blue in one tube and bright blue in the other; the deeper parts in each are gray (still reduced); 65th, casein blue, no purple in it, whey not red by reflected light.[1]	After 13th day, on the 15th, 2 to 3 mm. of whey on top of blue casein; 22d, casein has not solidified, but is slowly settling out as a blue finely divided precipitate. The visible whey is only 7 mm. deep.	After 13th day, partial on 22d and 27th; complete on 31st; casein gray; casein gray on 43d.	No acid reaction detected in either tube. Is peptone necessary?
1	442 (430+2½% cane sugar).	.....do	Good; excellent surface growth on 7th day.	1st, 2d, 3d days, no change; 6th and 7th, like check.	9th day, doubtful; if any change, not so blue; 13th, same color as	15th day, 9 mm. translucent whey on 45 mm. uniformly blue curd, which is a trifle paler than the check tube; 22d, whey nearly colorless,	After 9th day, on 13th, 1 mm. of whey on top of the uniformly blue	After 13th day, partial on 15th; 32d, casein pale blue; 43d,	Slight trace of acid at close.

					check. No red color.	casein blue, 3 cm. deep, not solidified, and settles very slowly; 27th, casein blue; 50th, color returning; whey pale brownish red, casein gray below, purple blue above; 65th, lower one-half of casein gray, upper one-half purple blue, whey pale purplish.	casein.	casein gray white, whey pale yellow; all reduced except a few pale blue flecks on yellow rim.	
1	443 (430+2½% galactose).	.....do	Good; excellent yellow surface growth on 7th.	1st, 2d, 3d days, no change; 6th and 7th, color like check.	9th day, no red; if any change in color, bluer; 13th, milk uniformly blue and slightly deeper than the check.	15th day, 3 to 4 mm. whey on top of the uniformly blue casein; color deeper than check; 22d, curd, uniformly blue, not solidified; about same color as check; as in all of the milk cultures the casein has separated out slowly as a voluminous, noncoherent precipitate; 27th, curd 3 cm. deep, blue, with a trace of purple in it; 50th, color oxidizing back; whey dull brownish red, upper one-half of casein deep hyacinth blue, lower one-half a dirty gray; 65th, casein hyacinth blue, whey dull wine red (reflected light).	After 13th day and before 15th.	After 13th day, partial before 22d (in whey); 27th, whey yellowish; 43d, whey pale yellow, curd grayish brown. No litmus color except in the yellow rim where there are many small pale blue patches.	Slight acidity in whey at close.
1	444 (430 + 1% maltose).	.....do	Good; copious surface growth on 9th day.	1st, 2d, and 3d days, no change; 6th, no change; 7th, a trifle lighter blue than check.	9th day, no red color, but a lighter blue than check; 13th, casein blue, but paler than check, not solidified.	15th day, slightly redder by reflected light than check tube; 22d, litmus in deeper parts of tube dull purplish blue; 27th, the litmus which is not reduced is pale purplish red by reflected light; 43d, fluid and casein dull purple; 50th, casein dull hyacinth blue; whey dull wine red (color is barely visible by reflected light); 65th, casein dark blue, no purple in it; whey dark by reflected light.	Very slight separation on 9th.	Doubtful, possibly some on 7th day; wholly reduced in upper 1 cm. on 22d; 27th, nearly reduced.	Slight acidity 3d and 4th weeks.

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[1] In stocks 441 to 446 (exclusive of 2d maltose test) the organisms were all destroyed on the 43d day by heating them 15 minutes at 80° C., falling to 62° C.

TABLE IX.—*Behavior of Ps. hyacinthi in litmus broths, and especially in litmus milk with and without alcohols and sugars*—Continued.

No. of tubes.	Stock.	Original color.	Growth.	Color first week.	Color second week.	Color third week and later.	Separation of casein begins.	Reduction begins.	Remarks.
1	444 (430 + 1% maltose); inoculated a month later from another culture.	Between lavender and royal purple.	Prompt; good. On 17th day, a wide, bright yellow rim.	4th day, bluer; 5th, deep blue—i. e., bluer than when inoculated.	8th day, same as on 5th.	17th day, casein blue (R's lavender, but twice as deep a color); whey not red by reflected light; 23d, fluid dull purple by reflected light; 38th, heated 10 min. at 56° C.; 49th, casein bluer than it was (litmus reoxidizing), whey dark blue by reflected light.	7th or 8th day (1 mm. whey on 8th).	Doubtful; casein grey blue and whey yellowish on 38th day.	Very slight indication of acidity on 23d day.
1	445 (430 + 2½% mannit).	.....do........	Good; distinct yellow pellicle on 4th day.	1st, 2d, and 3d days, no change; 6th and 7th, same color as check; no reddening.	9th day, no red color, doubtful if any change, possibly slightly bluer; 13th, fluid uniformly blue; slightly deeper than the check.	15th day, casein fills the greater part of the culture, and is a uniform opaque blue, deeper than the check; 22d, casein uniformly deep blue, and not coherent (solidified); it occupies all but upper 6 mm. of the culture; 27th, whey yellowish (reduced); most of culture occupied by the slowly settling casein, which is blue with a purplish tinge; 43d, fluid dull purple by reflected light; nearly all of the casein dissolved; 50th, casein dull hyacinth blue; whey dull wine red (reflected light); 65th, casein blue; whey dark (not red).	After 13th day and before the 15th, whey 4 mm. deep on 15th.	After 13th day. Partial in whey before 22d.	Slight indication of acidity on 27th, 43d, and 50th days.
1	446 (430 + 2½% c. p. glycerine).	.....do........	A good surface growth on 7th day; 22d, bacterial rim bright yellow, but not over one-tenth as much as in other tubes.	1st, 2d, and 3d days, no change; 6th, like check; 7th, slightly bluer than check.	9th day, slightly bluer than check; 13th, milk uniformly blue, distinctly deeper than check.	15th day, bluer than check; 22d, distinctly bluer than check; 27th, fluid uniformly blue; growth slower than in the other tubes (430–445); 31st, a decided dulling of blue, but no acid reaction; 50th, color returning; fluid uniformly deep blue; 65th, fluid deep blue, wholly opaque; distinct yellow rim and precipitate.	No separation of whey from the casein.	After 15th day. Partial on 31st; nearly complete on 43d.	Growth retarded by the glycerine; no acid formed.

2	532 c. p. litmus (same formula as 430, i. e., 10 c. c. of a saturated water solution of dry, lime-free blue litmus to 200 c. c. of milk).	.....do........	Good; a bright yellow pellicle 3d day.	2d day, purple rim above, cream has changed to blue; 3d, rim bluer; no change in body of milk; 6th, no red color, and no decided increase of blue; 7th, casein slightly bluer.	10th day, curd in one tube dark hyacinth blue, twice as deep as in checks; color in the other not quite so deep blue, but no indication of any acid; 13th, deeper blue than on 10th; one tube lags behind the other as if it had received fewer bacteria.	20th day, no indication of any acid reaction; litmus in the whey reduced, casein partly dissolved; unreduced litmus a dark plum purple.	Trace of whey (?) the 3d day; 4th, 2 mm. whey in one tube, 3 mm. in other; 7th, 7 mm. whey in one tube, 6 mm. in other.	Slight on 6th and 7th days, 10th, 11 mm. whey, of which four-fifths reduced in one tube and one-half in other; litmus in the casein not reduced.	No acid.

From an inspection of the foregoing table it is evident that, as a rule, under the conditions named, *Ps. hyacinthi* reduced litmus only very slowly. In litmus milk its first visible effect was a deeper bluing of the milk, which persisted for some time; the casein was then thrown down slowly, and a partial or complete reduction of the litmus usually followed. Upon reoxidation, the litmus was again blue. Addition of methyl alcohol led to no acid reaction. Addition of ethyl alcohol caused the development of a slight quantity of acid, which inhibited further growth, but did not immediately destroy the organisms. This acid is volatile and the boiling culture smells like acetic acid. Glycerin retarded growth, no acid was formed, and the casein did not separate. Addition of other substances to the litmus milk—e. g., mannit, galactose, cane sugar, grape sugar—led (during the first few weeks) either to the formation of no acid or to the production of so slight a quantity that it was easily obscured by the alkali.

Ps. campestris and *Ps. phaseoli* were also tested in litmus milk and other litmus cultures. In general, their behavior was like that of *Ps. hyacinthi*. The milk first became deeper blue, the casein was then thrown down slowly, and the litmus was reduced. In some cases, at least, the litmus was reduced more rapidly by these two organisms than by *Ps. hyacinthi*. On reoxidation the litmus was blue.

The relative rapidity of the reduction of litmus is worth noting. For instance, in some broths tinctured with this substance and inoculated with *Ps. hyacinthi*, all of the litmus color disappeared except in the uppermost layers in contact with the air, but this reduction took place slowly, requiring several weeks, where *Bacillus cloacæ* consumed only as many days.

In a litmus cauliflower broth inoculated with *Ps. hyacinthi* reduction was first visible toward the end of the second week and was not complete until after the third week. In the same broth inoculated with *Ps. phaseoli* reduction began to appear at the end of the first week and was complete at the end of the second week. In the same broth inoculated with an undescribed organism belonging to the *B. cloacæ* group[1] there was partial reduction of the litmus in 20 hours and complete reduction in 48 hours.

Ps. stewarti cultivated in the same litmus milk behaved differently. It grew well, but the casein was not thrown down and a slight amount of acid was formed. This is usually not observable the first week and it is often obscured for a long time by the reducing action of the organism. The action of this germ on litmus milk is shown in the following table:

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[1] Isolated from rotting potato tubers received from Florida and designated in the writer's notes as "The Florida gas-forming wet rot."

Table X.—*Behavior of Ps. stewarti in litmus milk.*

	Stock.	Color.	Growth.	Color changes.			Separation of casein.	Reduction.
				First week.	Second week.	Third week and subsequently.		
Ps. stew ..	373	Very deep lavender; deep as R.'s mauve, but lavender color.	Good.		9th day, no fading of color, no reddening; 13th, no visible change.	128th day, milk now lilac color, opaque; slime on wall out of fluid is 2 cm. wide and reddish yellow, i. e., dragon's blood or madder, with a dash of yellow.	No separation (128 days).	Reduced between 13th and 128th.
Ps. stew ..	373	.....do..........	34th day, considerable yellow precipitate.	No change for a number of days.		34th day, no reddening of fluid to date; color has slowly faded to a very pale lavender, much paler than R.'s No. 16, i. e., almost white, a copious yellow rim which begins to be reddish; 153d, fluid lilac, opaque; rim on wall out of fluid 1 cm. wide, reddish yellow.	No separation (153 days).	Slow bleaching of litmus, not visible for some days.
Ps. stew ..	394	Very dull hyacinth blue.			13th day, fluid deep purplish blue; no purple in corresponding tubes of *Ps. hyacinthi.*	17th day, more purple and less blue than on the 13th; color distinct from check and suggestive of deep prune purple; 27th, fluid purple-red.	No separation.	?
Ps. stew ..	399	Between hyacinth blue and plum purple.			10th day, very slight change from check, as if a trifle of reddening; 14th, a distinct change, i. e., a slight admixture of rose; color lies between mauve and heliotrope purple.	24th day, milk is now a uniform heliotrope color; the checks have remained unchanged; 31st, changes are going on very slowly; the milk is now a uniform dull heliotrope; on the bottom under the buff yellow precipitate the litmus is once more blue over a few sq. mm.; 48th, a centimeter breadth of red slime on wall out of fluid.	No separation.	No reduction to 14th day; 48th, litmus reduced to a dirty gray-white, with a trace of pink in it.
Ps. stew ..	400				10th day, blue, but redder than check; change slight; 14th, heliotrope purple.	24th day, a dull purple, approximating Indian purple; 48th, wide purple rim (bacterial) on walls above fluid.	Casein separated by excess of litmus in preparation of media.	No reduction on 10th day; 48th mostly reduced.

TABLE X.—*Behavior of Ps. stewarti in litmus milk*—Continued.

	Stock.	Color.	Growth.	Color changes.			Separation of casein.	Reduction.
				First week.	Second week.	Third week and subsequently.		
Ps. stew ..	430		Good; on 13th a copious yellow precipitate rim and pellicle.	1st to 6th day, no change; 7th, slightly bluer than check.	9th day, deeper blue than the checks; 13th, deeper blue than checks.	15th day, no reddening; 22d, color now very unlike that of check; over one-half the color is discharged, the tint now being a uniform pale lavender; 27th, color pale lavender except in bottom, where it is whiter; 32d, color lilac; 43d, lilac; 50th, color lilac; 65th, color now lies between lilac and heliotrope purple.	No separation (65 days).	None to 13th day; slight at bottom on 15th; on 22d, uniform slow reduction.
Ps. stew ..	532			2d day, purple litmus above cream has become blue; no other change; 3d, increased bluing of rim; 6th, fluid paler, but still lavender blue; 7th, rim purple, milk lavender, but paler or redder(?) than checks.	10th day, milk has slowly changed color; it is now heliotrope purple; 13th, as on 10th.	20th day, on the wall above the yellow pellicle the litmus is purple; milk pale, but not white or gray; it is a color belonging to Ridgway's series on Plate VIII; it came nearest to a mixture of lavender and lilac or lavender and heliotrope purple; the purple of the rim is deeper, i. e., more like Indian purple.	No separation.	On 6th day litmus uniformly paler than in checks; 13th, more reduction (at top).

TESTS FOR HYDROGEN SULPHIDE.

The tests for H_2S were made by suspending in the tops of test tubes, containing cultures of *Ps. hyacinthi*, narrow strips of filter paper which had been dipped in a saturated water solution of c. p. lead acetate. The strip was held in place by having its upper end wedged between the wall of the tube and the close-fitting cotton plug. The following trials were made:

(1) *Coconut culture.*—Growth good. Paper introduced on fourteenth day. Result: Strip feebly browned in 24 hours. Removed and inserted another moister paper. In 48 hours the lower 1 cm. of the strip was distinctly brown, but not deep brown.

(2) *Coconut culture (from another series).*—Growth good. Paper introduced on fourteenth day. Result: Very marked browning of the lead acetate paper in 48 hours. After 3 weeks the strip was black in lower one-half inch, and brownish for another one-half inch. The bacterial layer was bright yellow and the substratum unstained.

(3) *Carrot culture.*—Growth good. Paper introduced on the ninth day. Strip examined and remoistened on fifth day. Result: No browning of the paper so long as the experiment continued (42 days). Substratum browned.

(4) *Potato culture.*—Growth good. Paper introduced on fourteenth day. Result: No browning of strip so long as under observation (3 weeks). Substratum grayed. Fluid feebly browned.

(5) *Rutabaga culture.*—Growth good. Paper introduced on third day. Result: No browning of the lead paper in 47 days. A slow browning of substratum, and bacterial slime.

(6) *White radish culture.*—Growth good. Paper introduced on third day. Result: No stain of the strip in 61 days. Substratum browned.

(7) *Yellow globe turnip culture.*—Growth good. Paper introduced on third day. Result: Seventh day, copious growth; no stain of the lead paper. Eighteenth day, a slight browning of the strip at bottom and a feeble browning of the upper part of the substratum. Twenty-seventh day, a feeble browning of the lower part of the lead paper; distinct pale browning of the upper part of the substratum. Thirty-fourth day, a slow increase of the brown color in the lead paper; slime neutral. Sixty-fourth day, only a slight browning of the lower end of the lead acetate paper; substratum brown (burnt umber); fluid grown full (solid) with yellow-brown slime; reaction acid.

Conclusion: *Ps. hyacinthi* caused prompt browning of lead paper when grown on sulphur-bearing substrata, which did not stain brown. With one exception, there was no evolution of hydrogen sulphide (browning of lead paper) when grown on substrata which became gray or brown as a result of the growth of the organism, although some of these must have been very rich in sulphur compounds. Query: Was the H_2S fixed in the substratum as fast as formed, by ammonia, with the resultant brown stain? See The Brown Pigment.

Ps. campestris behaved in much the same way. The lead paper was promptly browned when exposed over cultures on coconut, and the substratum was not stained. Exposed over potato and rutabaga, there was no browning of the paper, but a brown staining of the substratum. Exposed over white radish and yellow globe turnip, on which growth was prompt and very copious, the paper browned slowly and the substratum also finally changed to brown. In a tube of radish there was no visible browning of the paper up to the fourteenth day of exposure, and on the same date there was only the merest trace of browning on the lower margin of the strip in the tube of yellow globe turnip. On this date there was an equally good growth in the 2 tubes, but there was no stain of the substratum in the tube of radish, while there was a distinct browning of the whole substratum in the tube of turnip.

Ps. stewarti grayed potato cylinders, but did not brown the lead paper (9 days' exposure). On rutabaga and yellow globe turnip it neither browned the paper nor stained the substratum (64 days). Also, on white radish in 64 days the substratum was not stained, but no test was made for H_2S.

Bacillus coli and an undetermined white organism (received as *B. coli* from the bacteriological laboratory of the Army Medical Museum), grayed potato cylinders promptly, but there was no browning of the lead acetate paper in 58 days.

For behavior of *Ps. phaseoli* see The Brown Pigment.

FORMATION OF INDOL.

The pink or red indol reaction was obtained with *Ps. hyacinthi* by adding sulphuric acid and sodium nitrite to cultures in Dunham's solution, in peptonized sugar-free beef broth, and in peptonized Uschinsky's solution. My practice was to add to the culture 15 drops of a mixture of sulphuric acid and water (2 acid, 1 water), and then 1 c. c. of distilled water containing 0.1 per cent sodium nitrite. If the color did not come at once, or within a few minutes (which was frequently the case), the tubes were plunged into water at 75° to 80° C. for 5 minutes, during which the color appeared. The color was a distinct red or pink. Uninoculated tubes tested at the same time gave no such reaction. Cultures of various ages were used, but none less than 3 weeks old. Old cultures must be used to obtain a distinct reaction, and in none was the color more than one-quarter as deep as that in corresponding tubes of *Bacillus coli*. In no case could any indol reaction be obtained from culture fluids which did not contain peptone. The same result was obtained with *B. coli* and a half dozen other organisms used for comparison. The presence in the culture fluid of peptone (using this term in the commercial sense) appears to be necessary for the production of indol.

The indol reaction was also obtained from cultures of *Ps. campestris*, *Ps. stewarti*, and *Bacillus amylovorus*.

TESTS FOR NITRITES.

Peptonized Beef Broths.

Two stocks were used: (1) A strongly alkaline beef broth (stock 382) with addition of 1 per cent Witte's peptonum siccum; (2) a slightly alkaline beef broth deprived of its muscle sugar by growing *B. coli* in it for 17 hours in the thermostat. This latter was clarified with the whites of 4 eggs, which were neutralized by HCl, and fortified with 2 per cent Witte's peptone. These cultures were tested on the twenty-second day after good growth. Neither gave any nitrite reaction with the indol-sulphuric acid test, the indol being that normally present in the cultures.

Peptonized Uschinsky's Solution.

This stock consisted of Uschinsky's solution with the addition of 1 per cent Witte's peptone. The tests were made at the end of 22 days. There was no nitrite reaction with the indol-sulphuric acid test, the indol being that normally present in the cultures.

Nitrate Bouillon (Stock 474).

This consisted of—

Distilled water, 1,000.0.
Witte's peptone, 10.0.
Beef extract, 2.5.
Chemically pure potassium nitrate, 3.0.

and sodium hydrate sufficient to render the fluid +10 of Fuller's scale.

Ps. hyacinthi grew readily in this medium without gas production. Examinations for nitrite were made on the sixth, sixteenth, and twentieth days, using the iodine-starch test—i. e., to each tube was added 1 c. c. of thin boiled starch water, 1 c. c. of one-half per cent potassium-iodide water (which should be freshly prepared), and finally a few drops of a fluid consisting of 2 parts of c. p. sulphuric acid and 1 part of distilled water. No trace of nitrite reaction could be obtained with this reagent. Subsequently grape sugar was added to some tubes of this nitrate bouillon (100 milligrams per 10 c. c.), but even in the presence of this agent *Ps. hyacinthi* was unable to reduce any nitrate to nitrite (8 days). Tubes of *Bacillus coli* and of *Bacillus carotovorus* were used for comparison. These became blue-black, like ink, on addition of the sulphuric acid.

Ps. campestris and *Ps. stewarti* resemble *Ps. hyacinthi*. Neither one is able to reduce potassium nitrate to nitrite in peptonized bouillon cultures, either with or without grape sugar. Comparisons were also made with *Bacillus amylovorus* and *B. pyocyaneus pericarditidis*. The former does not reduce nitrates to nitrites. The latter (like various other green-fluorescent bacteria) first converts the nitrate to nitrite, and then liberates the azote as free nitrogen.[1] Gas bubbles were given off continually during the first few days, so that the top of the fluid was foamy, as if it had been shaken violently. During this stage the liquid gave a deep blue-black reaction with boiled starch water, potassium iodide, and sulphuric acid. Later the gas bubbles disappeared, and then (sixteenth day) no nitrite reaction could be obtained. The experiment with *B. pyo. pericarditidis* was repeated, using fermentation tubes; a considerable quantity of gas collected in the closed end. This gas was not absorbed on shaking with caustic soda (absence of CO_2); it did not diffuse quickly or explode when it was tilted into the open end of the tube and a lighted match applied (absence of hydrogen and marsh gas); lighted matches thrust into the bowl were repeatedly extinguished (presence of nitrogen).

One or two other interesting facts were observed in connection with cultures in the nitrate bouillon. *Ps. stewarti* and *B. amylovorus* made a very feeble growth in comparison with *Ps. hyacinthi* and *Ps. campestris*. *B. coli* grew better, throwing down in 16 days about 10 times as much precipitate as *B. amylovorus*. In early stages of growth, i. e., during the first 2 or 3 days, the 4 cultures of *Ps. campestris* were very different from those of *Ps. hyacinthi* in that the former contained many hundreds of tiny white zooglœæ scattered uniformly through the liquid, giving it, especially under the Zeiss × 6 aplanat, a distinctly granular appearance. On the sixteenth day this phenomena had disappeared and the cultures of the two organisms were then much alike.

Ps. phaseoli was also tested in this nitrate bouillon. Like *Ps. campestris*, it formed great numbers of small zooglœæ during the first few days of growth. It was entirely unable to reduce the nitrate to nitrite in this solution (14 days).

FERMENTS.

No attempt has been made to isolate any ferment, but the behavior of *Ps. hyacinthi* in the host plant and in various culture media leads to the conclusion that several enzymes are secreted.

CYTASE.

The thin, non-lignified walls of the spiral vessels of the host plant are dissolved, letting the bacteria out of the vascular system into contact with the parenchyma. Fragments of the spiral threads are apparently all that remain of these vessels in bundles which have been long occupied. Once in contact with the parenchyma, cavities, filled by the bacteria, are formed in this tissue, the cells being first separated from each other and finally destroyed, as Dr. Wakker has described. These facts indicate the secretion of a cytohydrolytic enzyme. At the same time the slowness with which the vessels are destroyed and the cavities formed lead me to think that this substance is secreted only in extremely small quantities. The results of growth on different vegetable culture media point to the same conclusion. No softening of the cell walls was observed in any of the following substrata: Potato, sweet potato, sugar beet, coconut. A softening of the middle lamella of carrot, turnip, and radish cylinders was noted in old cultures.[1]

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[1] These are the organisms that reduce the value of the farmer's manure pile.

A few observations were made on the related organisms. Potato, coconut, rutabaga, yellow globe turnip, and radish cylinders were not softened by *Ps. stewarti*. *Ps. campestris* softened cylinders of potato, rutabaga, and yellow globe turnip.

The behavior of *Ps. campestris* in the interior of various host plants, in the absence of any other organism, indicates that a cytase must be present, i. e., closed cavities are formed. During the formation of these cavities, which are fully occupied by the bacteria, the parenchyma cells are first separated from each other by a multiplication of the organism in the intercellular spaces, the walls of the cells are then crushed together by the continued multiplication of the bacteria, and become more and more indistinct, until they finally disappear altogether.

In properly fixed, paraffin-embedded material, cut in serial section, all stages of the solution of the cells and the formation of these bacterial cavities may be readily observed, especially in the easily sectioned cabbage and turnip occupied by *Ps. campestris*. The organisms find their way into the parenchyma from the vessels, which are first occupied in ways already described by the writer elsewhere. That the destruction of the cell walls can be due to nothing but this organism, in the disease under consideration, is shown clearly as follows: (1) Because these are closed cavities, i. e., not in open connection with the surface of the plant, except at long distances from the place of occurrence; (2) because these cavities occur as freely in the interior of plants that have become diseased from the writer's pure-culture inoculations as they do in those which have become diseased naturally in the fields; (3) because the microscope shows the cavities to be filled exclusively by bacteria; (4) because cultures made from the interior of such inoculated and diseased plants have shown *Ps. campestris* to be the only organism present; (5) because all stages in the destruction of the cells and in the formation of these cavities can be followed in serial sections, so fixed and otherwise prepared that the relation of the bacteria to the various parts of the host plant is the same as in the living plant.

Ps. phaseoli also forms cavities in the interior of the host plants. Concerning *Ps. stewarti* I am in doubt.

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[1] Since this was written, and too late to determine experimentally, it has occurred to me, as the result of reading Potter's papers, that possibly this solvent action on the middle lamella is due to the formation of *acid ammonium oxalate*. It cannot be due to oxalic acid as such since this has no solvent action on turnip tissues.

INVERTASE.

A slant tube of 10 per cent cane sugar agar, fragments of which gave no precipitate of copper oxide on boiling 2 minutes in Soxhlet's solution, gave after *Ps. hyacinthi* had been grown on it for 29 days, a very copious rusty precipitate after boiling 2 minutes in the same solution. Cane-sugar bouillon gave the same result. This indicates that cane sugar is inverted, and to a much greater extent than is needed for the growth of the organism, but we may not therefore assume the existence of an invertase. The fact that cane sugar was not inverted when put into dead or sterile tubes of *Ps. hyacinthi* cultivated in beef broth and peptonized beef broth, seems to show either that the living organism itself is necessary to bring about the inversion or else that invertase is formed only when it is required, i. e., in the presence of cane sugar.

My first experiments were in non-peptonized alkaline beef broth (stock 382). The contents of tubes 3, 4, 7, 10, 12, and 15 of February 7 (cover-glass inoculations 24 days old) were poured together and forced through a Chamberland filter. Two 10 c. c. portions of the sterile fluid were then pipetted into cotton-plugged sterile test tubes, and to each was added 300 milligrams of cane sugar. To one of these tubes chloroform was added and to the other thymol. They were then set away at 18° to 24° C.

On the fifth day each tube was tested by pipetting 2 c.c. of the clear fluid into boiling Soxhlet's solution, and continuing the boiling 1½ minutes. In neither case was there any reduction. These tests were repeated on the thirty-fifth day with the same negative result.

A duplicate series from tubes 1, 8, 14, and 18 of February 7 (same stock) led to the same result. In neither portion was there any reducing sugar on the fifth or thirty-fifth day.

Thinking that the invertase might possibly have been retained in the walls of the filter, or that the presence of peptone might be essential to the formation of invertase, the experiment was repeated as follows:

Three old cultures of *Ps. hyacinthi*—(1) in beef broth with Wittes's peptone (459); (2) in beef broth without peptone (382), and (3) in beef broth with the trace of muscle sugar removed by *B. coli* (404)—were sterilized by heating them for 10 minutes at 54° C., viz, at a temperature high enough to kill the organism and low enough to be harmless to invertase. To each tube was then added 500 milligrams of cane sugar and 150 milligrams of thymol. The sugar was transferred from a sterile solution by means of a sterile pipette. Along with these three cultures two other old cultures were tested, viz, one of *Ps. campestris* and one of *Ps. stewarti*, each in stock 382. These tubes were set away for 19 days at 25° to 30° C.

At the end of this period they were tested as follows for the presence of reducing sugars:

Twenty-five cubic centimeters of Soxhlet's standard alkaline solution was added to 25 c. c. of his standard copper sulphate solution, and after mixing was divided into 5 equal parts in 5 clean porcelain capsules and 40 c. c. of distilled water added to each one. The fluid in one of these capsules was then brought to a boil and 1 c. c. from one of the tubes was added to it and the boiling continued for 1½ minutes. In the same way each of the other tubes was tested. In none of the 5 capsules was there any reduction of the copper.

A more conclusive test would be to grow these organisms in sugar bouillon for some weeks and then determine per cubic centimeter the exact copper-reducing power of the cultures. These should then be heated 10 minutes at 54° C., or thereabouts—i. e., long enough to destroy the organisms. Thereupon, measured volumes should be pipetted into sterile cane-sugar solutions. To similar solutions should be added equal portions from the cultures after heating them for 10 minutes at 80° or 90° C.—i. e., long enough to destroy the supposed invertase. Then after some weeks, if the fluids have remained sterile, their reducing powers should be determined quantitatively. An experiment of this sort was begun with *Ps. hyacinthi*, but was lost through a contamination which was probably introduced with the thymol. At least the intruding white organisms were capable of growing in the presence of an abundance of this antiseptic at a constant temperature of 50° to 52° C.

As the writer has had no opportunity to repeat the experiment, the question of an invertase must be left an open one. This only is tolerably certain—none is formed in the absence of cane sugar.

All of these 4 yellow organisms invert cane sugar readily, as already pointed out.

DIASTASE (AMYLASE).

The experiments with starchy media, already described, show that the diastasic action of *Ps. hyacinthi* is very feeble. Nevertheless, some growth occurred, even when the greatest care was taken to exclude all carbohydrate food except pure starch; and as tests with iodine water and with Soxhlet's solution showed that there had been a slight action on the starch, minute quantities of a diastatic ferment must be secreted. The starch which has been acted upon gives the red or amylodextrine reaction with iodine. *Ps. stewarti* acts on starch slowly, after the manner of *Ps. hyacinthi*.

On the contrary, *Ps. campestris* and *Ps. phaseoli* destroy starch and amylodextrine promptly in considerable quantities, so that in course of a few weeks none, or very little, is left in the culture tube, even when there were several grams of starch at the outset.

Experiments with both *Ps. campestris* and *Ps. phaseoli* showed that starch was converted in the absence of the bacteria (tubes heated for some minutes at a few degrees above the thermal death point and some of the fluid then added to potato starch with antiseptic precautions) and that none was converted if before adding them to the starch the fluids were heated to a point above that at which diastase is destroyed.

TRYPSIN.

A peptonizing ferment must be present, since gelatin and Lœffler's solidified blood serum are liquefied, and casein is slowly dissolved with the formation of tyrosin. This, also, is secreted in minute quantities or else is partially inhibited by other substances, because gelatin is liquefied very slowly even under favorable conditions—i. e., optimum temperature, proper alkalinity, and suitable food.

Ps. campestris and *Ps. phaseoli* behave in the same way—i. e., they liquefy gelatin and Lœffler's solidified blood serum and dissolve (peptonize) casein. These processes take place more rapidly than in case of *Ps. hyacinthi*, but in none of them is the peptonization speedy. *Ps. stewarti* does not liquefy gelatin or Lœffler's solidified blood serum.

LAB FERMENT.

The existence of a lab or rennet ferment is at once suggested by the fact that in milk cultures the casein is thrown out of solution in the absence of any visible production of acids (see Milk and litmus milk and Litmus under reduction experiments). The casein is also precipitated if the whey from old alkaline milk cultures is first sterilized by heating it for ten minutes at 56° C. and then added to tubes of sterilized milk along with thymol. Media inoculated from the thus coagulated milk remained sterile.

The same whey, after heating for ten minutes at 90° C., had no effect upon milk.

Ps. campestris and *Ps. phaseoli* behave in the same way. Both throw down casein by means of a lab ferment. *Ps. stewarti*, on the contrary, produces no lab ferment and never coagulates milk.

OXIDIZING ENZYMES.

No oxidase or peroxidase was detected—i. e., the cultures of *Ps. hyacinthi* did not react blue with sensitive guaiac resin in alcohol nor was there any bluing on the subsequent addition of hydrogen peroxide. *Ps. campestris* behaved in the same way. The brown stain is believed to be due to other causes.

A copious evolution of gas bubbles took place when hydrogen peroxide was added to old potato cultures of *Ps. hyacinthi*, *Ps. campestris*, *Ps. phaseoli* and *Ps. stewarti*.

Such copious evolution of oxygen is, however, not peculiar to these particular parasites. It has been more recently observed by the writer in case of old potato cultures of *Bacillus coli*, *B. amylovorus*, *B. pyocyaneus pericarditidis*, a fluorescent germ obtained from fermenting tobacco and able to grow in the presence of thymol, Earle's bacillus of tomato fruit rot, an orange colored clumpy organism from cotton leaves, a dendritic yeast, and a nondendritic yeast (both yeasts were obtained from the sticky surface of Niagara grapes). In all of these cases the gas soon foamed over the top of the test tube. An old coconut culture of *Ps. hyacinthi* also gave a very considerable quantity of gas.

The least amount of gas was obtained from adding H_2O_2 to 3-months-old potato cultures of Jones' carrot rot bacillus. Three tubes were tried, all of which behaved alike. At first there was no gas, then a slow, long continued evolution of small bubbles, the total not being very great. A young potato culture of this bacillus (8 days old) yielded gas almost from the start and in much greater quantity than the old cultures. The reverse was true of *Ps. campestris.* A potato culture 3 months old yielded gas more promptly and in greater volume than did a vigorous culture made from the same tube and only 8 days old. Both, however, yielded much gas. On the contrary, even that from the young cultures of Jones's bacillus fell far behind in amount that which was evolved by the other 10 bacteria and by the two yeasts.

Some gas was also obtained by pouring H_2O_2 upon old rice cultures of various fungi, e. g., *Fusarium niveum*, *F. vasinfectum*, Swingle's Atta fungus (cultivated by the writer from a nest of Atta, near Washington), and from an agar plate culture of cotton anthracnose.

The yield of gas from the fungi named was insignificant in comparison with that obtained from the yeasts and from the bacteria, exclusive of the old cultures of Jones's bacillus. The other bacilli and the two yeasts gave so much gas that the tubes were filled and frothed over, usually within a few minutes.

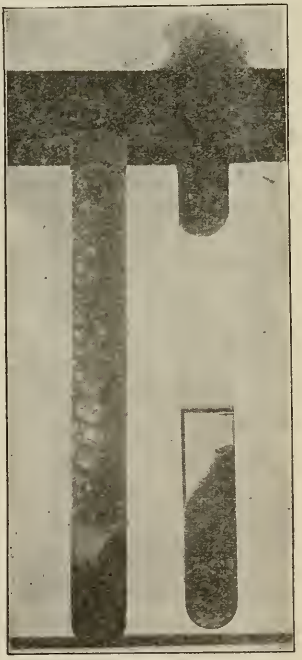

FIG. 1. (*a*) Evolution of gas on adding hydrogen peroxide to potato culture of *Ps. phaseoli;* (*b*) Uninoculated tube, to which hydrogen peroxide has just been added.

In the accompanying illustration (fig. 2) a 3-months-old potato culture of *Ps. phaseoli* to which H_2O_2 has been added is shown by the side of a check tube (uninoculated) to which H_2O_2 has also been added. In the one case there was a very copious evolution of gas bubbles, which filled the tube; in the other there was only a very slight evolution of gas, which soon ceased.

On heating a 3-months-old culture of *Ps. phaseoli* for 25 minutes at 75° to 85° C., and then adding the H_2O_2, there was no evolution of gas at first, but after a few minutes bubbles began to be given off and a small amount of froth collected, but not over one five-hundredth as much as from the unheated tube. This tube was, of course, full of a thick yellow slime, which perhaps conducted heat badly.

In a second test, a similar potato culture of *Ps. phaseoli* was exposed for 2 hours at 85° C. On then adding H_2O_2 there was no evolution of gas either immediately or after a time. A similar potato culture of *Ps. campestris*, treated in the same way, behaved the same—there was no evolution of gas. The above illustration would serve equally well for the behavior of tubes of *Ps. campestris* or *Ps. phaseoli* before and after heating to 85° C.

As already shown, both *Ps. phaseoli* and *Ps. campestris* when grown on potato produce an abundance of diastase, but the breaking up of the H_2O_2 with liberation of oxygen can hardly be due to that enzyme, for a potato culture of *Ps. hyacinthi* of the same age as the preceding gave an enormous quantity of gas, although, as usual, it had made a rather meager growth (owing to its feeble diastasic action). This potato gave a strong starch reaction with iodine potassium iodide. Stearns & Co.'s pancreatic diastase also failed to cause any evolution of gas when it was dissolved in water and H_2O_2 added.

Dr. Oscar Loew has given reasons for believing that this decomposition of hydrogen peroxide is due to a hitherto unsuspected oxidizing enzyme, which he has named *catalase*,[1] and which he believes to be universally distributed in plants and animals and to have to do with respiration.

PIGMENT STUDIES.

The Yellow Color.

Dr. Wakker appears to have been uncertain whether the yellow color was inherent in the organism itself or only in a gummy substance surrounding it.

The yellow color of *Ps. hyacinthi* can not be shaken loose or filtered away from the bacterial cells by water, and, with the exception of nutrient starch jelly containing glycerine, it was never imparted to any of my fluid or solid culture media, whether neutral, acid, or alkaline. It pertains only to the bacteria themselves. Working in a good light with the best appliances at my disposal, viz, Zeiss 2 mm. apochromatic, 1.40 n. ap., with 12 and 18 compensating oculars, it has never been possible to locate the yellow pigment in any gum or granules lying between the cells. In my opinion the color is lodged *within*

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[1] (1) Physiological Studies of Connecticut Leaf Tobacco. Department of Agriculture, Washington, D. C., 1900; (2) Catalase, a new enzyme of general occurrence, with special reference to the tobacco plant. Report No. 68, United States Department of Agriculture, Washington, Government Printing Office, 1901, pp. 47.

the organism, and is insoluble in water because it is dissolved in an oil secreted by the cells. The small size of the rods and the minute quantity of pigment in each one, has made it impossible to decide whether the color is lodged in the cell wall or in the cytoplasm itself. In whichever place, it appears to be uniformly distributed.

The intensity of the color depends, of course, on the density of the growth and also to some extent on its age and on the nature of the culture medium. It is always a distinct yellow. In the host plant it is chrome yellow to pale cadmium. It is also bright yellow on many culture media, especially when grown in the dark, e. g., gamboge, chrome yellow, or canary yellow. Occasionally, in cultures, it has been as pale yellow as primrose or maize yellow, but this has been the exception. and in some of these very pale cultures many involution forms were present. On some media, but not on all, old cultures became brownish or dirty yellow. In such cultures the slime has been dull yellow. dirty yellow, dark yellow, brownish yellow, ochraceous, and between ocher yellow and tawny olive. In young cultures, and in old cultures in which the brown stain was not detected the following shades of yellow have been seen: Primrose, maize, Naples yellow, wax yellow, gallstone yellow, saffron yellow, buff yellow, Indian yellow, gamboge, chrome yellow, deep chrome, lemon yellow, and canary yellow. The color was very dull in potato broths, but the whitish rim from such tubes made a homogeneous bright yellow growth when rubbed on suitable culture media. The color was also dull yellow in acid (unneutralized) beef broths, but in alkaline (soda) ones of the same origin it was bright (canary) yellow. The color was bright yellow in strongly alkaline gelatin and also in cane-sugar gelatin which had been acidified with malic acid. Excess of malic acid in the gelatin appeared to favor the development of the color, it being decidedly brighter in +54 than in +36 gelatin. The color did not appear to be any brighter when the organism was grown in the ice chest at 8° to 12° C. than when grown (in an equally dark place) at room temperatures of 25° to 30° C.

This color appears to be an oxidation product. It forms abundantly only in organisms near the surface of solid and fluid cultures. It is bleached by reducing agents, and regains its color after these have been removed. It does not form so abundantly when the organism is grown on suitable media in air containing a considerable reduction of free oxygen, i. e., on potato or coconut in nitrogen or carbon dioxide mixed with air. In these gases, when pure, there is no growth. In partial vacuum growth is less abundant and the color is paler yellow than in air. The same is true in nitrogen containing some oxygen, i. e., in air with the oxygen incompletely removed. (See Aerobism.)

The following substances bleach this color: Sulphuric ether, chloroform, turpentine, benzine, benzole, xylol, toluol, carbon bisulphide (contaminated with H_2S),[1] and nascent hydrogen. The loss of color was most rapid in the carbon bisulphide, 30 to 60 minutes sufficing, in some cases, to render the bright yellow bacterial slime as white as white lead. On removing this fluid, which was neutral to litmus, but the vapor from which browned lead acetate paper, the yellow color began to return in a few hours and finally became nearly as bright as before. The other substances reduced the color more slowly, and on their removal it was a much longer time in coming back, and never became quite as bright as at first. The test with hydrogen was made as follows: The pigment was extracted by 23 days' exposure to c. p. glycerin. Into this yellow glycerin was then thrown a small scrap of zinc and some 30 per cent c. p. HCl., which caused a continual evolution of gas. On the sixth day the yellow color was still visible; on the seventh day it was nearly gone; on the tenth day it was all gone. On the thirteenth day the zinc was removed from the now colorless fluid. The fluid remained colorless for some days (a week or two). It then very gradually changed to yellow; 54 days after the removal of the zinc it was still only feebly yellow. The yellow color was not dissolved out by any of these reducing substances; at least no yellow stain was imparted to the fluids. The bacteria were hardened by alcohol, ether, and chloroform into tough masses not easily divided. Similar masses remained soft under xylol, toluol, and turpentine, and had an unctuous feeling when touched with a glass rod. Owing to the hardening action of chloroform, the writer uses it in preference to turpentine or xylol in passing the tissues from absolute alcohol into paraffin. In sections cut therefrom the tissues of the host plant will tear or become displaced more readily than the bacterial sheet.

This pigment is slowly soluble in glycerol, as Wakker pointed out. It is also soluble in water containing hydrogen peroxide, in ethyl and methyl alcohol, in acetic ether, and in acetone. The latter proved the most ready and satisfactory solvent, most of the color being removed in 30 to 60 minutes. Ethyl and methyl alcohol and ethyl acetate are rather slow and feeble solvents. The color is slowly soluble, without destruction, in strong ammonia water; it is quite soluble in water saturated with ammonium carbonate. It is also soluble on long standing in glacial acetic acid. It is insoluble in petroleum ether. It was not dissolved or changed by remaining 30 days in $\frac{N}{10}$ HCl. The color was not destroyed by steaming 25 minutes in water, nor by boiling in strong ammonia water. It was not reduced by steaming in water containing grape sugar.

The acetone extract appears to be sensitive to light. On exposing

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[1] This is the carbon bisulphide which was used in my experiments with *Ps. campestris* (Centralb. f. Bakt., 2 Abt., Bd. III, page 479).

40 c. c. of the yellow acetone extract for some hours to bright sunshine on a tin roof, at 50° to 60° C., the fluid was reduced to 1 c. c., but instead of being an intense yellow, as was expected, it became a very pale yellow—i. e., there was less yellow in the 1 c. c. remaining than in the same quantity of the original fluid.

This color is not an oil, but seems to be intimately associated with such a body. On evaporating a yellow acetone extract (organism grown on sugar beet) to one-tenth or one-twentieth of its volume, the perfectly clear fluid became whitish cloudy, like an emulsion, and on examining it under the microscope it was seen to be composed of innumerable round bodies having the optical and chemical properties (osmic acid test) of oil globules. The yellow color was visible where these globules were massed, and also in noncrystalline patches, but separate oil globules did not appear to be yellow.

On driving off the remainder of the acetone with gentle heat, a small amount of chrome yellow, oily looking and oily feeling fluid remained in drops on the bottom of the white capsule. On adding concentrated c. p. H_2SO_4 to these yellow wet-shining drops there was an immediate decided blue-green reaction, which quickly changed to brown and soon after to brown-purple. After one-half hour an oily looking rim of brown-purple granules surrounded the drops of acid. This purple color was also fugitive, fading to a dirty gray. The acetone which was used changed to a clear brown on adding c. p. H_2SO_4, but with no preliminary blue-green color. The yellow residue which remained on evaporating the acetone extract from another lot of cultures (organism grown on coconut) changed at once, on adding concentrated c. p. H_2SO_4, into a green, which soon faded to purple. On adding the acid directly to the bacterial slime dried on glass slides it became orange-brown, then rusty-brown, and finally rose-brown, but no green or blue color appeared.

The presence of highly organized nitrogenous bodies is not necessary to the formation of this color. It is produced readily in Uschinsky's solution, with starch substituted for glycerin, and on this medium the yellow color is as pure and as bright as it is in the host plant or on coconut, sugar beet, peptone agar, or sugar gelatin.

These results lead me to think that this yellow color belongs to the Lipochrome group of plant pigments. (See Zopf: Die Pilze, p. 144.)

So far as I have tested it, the yellow pigment of *Ps. campestris* behaves in the same way, i. e., it is soluble in glycerin, ethyl alcohol, methyl alcohol, acetone, ammonium carbonate in water, and glacial acetic acid; it is insoluble in sulphuric ether, chloroform, xylol, toluol, or carbon bisulphide, but is bleached by these substances. As a rule, the yellow color of *Ps. hyacinthi* is brighter than that of *Ps. campestris* or *Ps. phaseoli*.

The Brown Pigment.

Under certain circumstances, not clearly understood, a pale brown pigment, soluble in water, is also produced by *Ps. hyacinthi.*

This feeble brown stain occurs in the host plant; in hyacinth broth; in alkaline peptonized beef broth (after 5 or 6 weeks); in one-half strength potato broth with 1 per cent Witte's peptonum siccum (not when the peptone is omitted); in the same peptone potato broth with addition of malic acid; on radishes (49 days, not in 25 days), white turnips, and yellow turnips; on banana pulp and banana rinds; and in water surrounding potato cylinders, the potato itself being grayed.

This pigment did not appear in any of the following media, not even in very old cultures: Acid beef broths (55, 59, 75, 80 days); alkaline beef broths free from peptone (33, 67, 71, 97, 100, 119 days); alkaline beef broth with cane sugar (26, 39, 67, 82, 98 days); distilled water with 4 per cent maltose, 4 per cent dextrine, and 4 per cent Witte's peptone (29, 40 days. Doubtful at the end of 70 days—no brown stain in one tube and a slight (?) browning in the other); 4 per cent peptone water (15 days); one-half strength potato broth (73 days); the same with small amounts of caustic soda (59, 73 days); Uschinsky's solution (83 days); standard agar containing some muscle sugar, acidity +22 of Fuller's scale (22 days); standard agar containing no muscle sugar, acidity +15.5 (13, 18, 47 days); the preceding agar with grape sugar (18, 29, 47 days); the same with cane sugar (29, 47 days); the same with fructose (31 days); litmus alkaline gelatin (39 days); malic acid gelatin (34 days); malic acid gelatin with cane sugar (174 days); gelatin neutral to phenolphthalein with soda (87 days); the same with cane sugar (61 days); sugar beet (55, 60, 67, 70 days); coconut (49 days, 95 days); potato with cane sugar (2 tubes, 67 days—a third tube showed slight browning on sixty-seventh day, but less than tubes without the sugar); nutrient starch jelly made from Uschinsky's solution by substituting washed potato starch for the glycerol (35, 62 days); the same with Taka diastase (39, 62 days); the same with maltose (30 days); the same with dextrine (30 days).

In the inoculated hyacinth plants the brown stain was not very noticeable, being confined, so far as observed, to the vascular bundles of the diseased leaves, and easily overlooked. In nutrient media the pigment does not appear immediately and is best observed in old cultures (1 to 3 months). It is never as pronounced, either in the host plant or on culture media, as the similar pigment formed by *Ps. campestris.* The most decided browning was in old cultures on cruciferous substrata and on banana skins. My failure to obtain any browning in gelatin cultures contradicts Dr. Wakker's statements, but this contradiction may be apparent rather than real—i. e., dependent, possibly, on differences in the chemical composition of the nutrient gelatins employed. On the other hand, it is probable that the browning he observed arose from the presence of some intruding organism—e. g., the one which produced gas bubbles in his gelatin.

The following shades of brown were observed: (1) A slight browning (yellow banana pulp, 55 days; water around potato cylinders, 31 days); (2) feeble brown (white turnip, 22 days; water around potato cylinders, 24, 37, 67 days; washed potato starch cooked in distilled water with 4 per cent Witte's peptone, 73 days; hyacinth broth, 59 days); (3) pale brown (yellow turnips, 23 days; one-half strength potato broth with 1 per cent Witte's peptone, 41, 59, 73 days; the same with malic acid, 41, 73 days); (4) brownish (a potato cylinder with 500 mgs. Merck's diastase of malt absolute, 41 days); (5) feeble reddish brown and later brownish white with the slightest trace of pink (washed potato starch with 4 per cent peptone water and Taka diastase, 44, 73 days; also the same stain without the diastase but feebler); (6) tawny olive (white turnips, 40 days); (7) paler than tawny olive (yellow turnips, 22 days); (8) ochraceous (white radish, 50 days); (9) russet (white turnips, 40 days); (10) between russet and burnt umber (yellow turnips, 40 days); (11) light burnt umber (white turnips, 49 days); (12) midway between burnt umber and mummy brown (yellow globe turnip, 50 days); (13) burnt umber (yellow globe turnip, 64 days); (14) sienna with a very slight admixture of brown (radish, 49 days); (15) dark brown (skin of yellow banana, 56 days).

The formation of this pigment appears to depend on the presence of certain highly organized nitrogenous bodies—e. g., albuminoids or peptones. Whether it is produced inside of the bacterial cell and dissolved out, or is formed in the substratum by the chemical action of colorless substances excreted from the cells, as seems more likely, could not be determined. Its formation also appears to depend on the presence of free oxygen, as in one instance, in an old culture on rutabaga (50 days) it was observed that the upper part of the substratum was distinctly browned, but in that part protected from the air (the lower one-half of the cylinder, in water grown full of the yellow slime and solidified) it was not browned.

Ps. stewarti grayed potato cylinders, but in two months it formed no brown pigment in tubes of radish, rutabaga, or yellow globe turnip. In from 6 weeks to 2 months *Ps. campestris* stained these same cruciferous substrata various shades of brown—e. g., (1) raw sienna, (2) a color between russet and cinnamon rufous, (3) a color between russet and tawny olive, (4) raw umber, (5) burnt umber, (6) dark burnt umber, (7) mummy brown.

These brown pigments are also believed to be in some way connected with the presence of sulphur compounds and of tannins or related bodies in the plant or substratum, and with the formation of hydrogen sulphide and ammonia by the bacterial organism.

As we have already seen, H_2S is given off promptly from coconut cultures of *Ps. hyacinthi* and *Ps. campestris*, which do not become grayed or browned, and is not given off from potato or carrot cultures, which do become stained.

A graying of steamed potato cylinders with subsequent pale browning of the water in which they stand—viz, a change similar to that induced by many different sorts of bacteria—is readily produced by adding to the tubes a few drops of ammonium sulphide. Tannin, in the air, is oxidized readily to deep brown compounds when exposed to ammonia, but this change does not take place in vacuo neither did the potato cultures gray in vacuo. All bacteria or nearly all produce ammonia and hydrogen sulphide, and many vegetable substances contain tannins or allied compounds.

A somewhat different result was obtained with *Ps. phaseoli*, which also grays potato cylinders and becomes dulled in color by the formation of a small amount of soluble brown pigment. My attention was drawn especially to this by the behavior of some potato cultures. Eight of these were alike in their yellow color and the substratum was grayed; the ninth, while alike in all other cultural respects, was a much brighter yellow, and there was no distinct stain of the potato. At the time I had in the laboratory two stocks of potato made from different tubers. The question now arose whether the bright chrome yellow culture was specifically different from the wax yellow cultures, or had been made accidentally on the newer potato stock and was the same species, but different in color on account of some slight chemical difference in the culture medium. To test this latter hypothesis a tube from each potato stock was now inoculated from the bright yellow culture. In one of these daughter tubes the growth was dull wax yellow, and the substratum was distinctly grayed within 48 hours. In the other the equally abundant growth was bright chrome yellow, exactly like the culture from which it was made. There can be no doubt, I think, that the usual dulling of the slime of *Ps. phaseoli* on potato is to be ascribed to the absorption of a brown pigment formed out of some substance commonly present in the substratum. These two cultures made from the bright yellow culture were tested for H_2S on the fifth day by placing strips of lead acetate paper in the top of the tubes under the cotton plug. The paper in the dull yellow culture browned promptly. That in the bright yellow culture did not brown at all at first (24 hours), but finally browned feebly, corresponding to a slowly appearing feeble gray color in the substratum. When the cultures were 9 days old and the paper had been exposed 4 days the conditions were as follows: The tubes were alike in volume of growth and in general appearance, except as given below. In one the color was a dull wax yellow, the lead paper was dark brown at the lower end, the substratum was distinctly grayed, and the bacterial slime reacted immediately and distinctly alkaline to neutral litmus paper. In the other tube the color was bright yellow (gamboge), the lead paper was feebly browned (only about one-tenth as much as in the preceding), the substratum was very feebly grayed, and the bacterial slime reacted differently to the neutral litmus paper—i. e., it was exactly neutral.

Ps. phaseoli cultivated on yellow and white turnips made a good growth, but no brown pigment was observed. On turnip-rooted radishes the growth was also good and there was no brown stain for a month, but after that a slight stain appeared.

NATURE OF THE CELL WALL.

The bacterial cell wall of *Ps. hyacinthi* stains yellow with iodine potassium iodide, and remains yellow on the addition of sulphuric acid (Russow's cellulose test). Tests were made with germs grown on agar, potato, starch jelly, etc.

The bacterial slime from cultures on banana and sweet potato reacts blue with Russow's test. At first this was supposed to indicate cellulose in the bacterial wall. Subsequently it was discovered that the blue reaction is due to some substance which may be washed away in water, the bacterial masses then giving only a yellow stain. This substance, which reacts blue, is believed to form no part of the bacterial cell, but to be the dissolved substances of the substratum, carried up and held between the bacterial cells by capillarity. These experiments were repeated a year later with banana, using old cultures which bore a thick, dull yellow slime. This slime gave no blue reaction with iodine potassium iodide (absence of starch), but a very decided blue on adding sulphuric acid. Several washings in water greatly reduced the tendency to this blue reaction, but did not entirely prevent it. This was believed to be due to the fact that the water had not penetrated into the center of all the bacterial masses. The experiment was therefore repeated as follows: Masses of the surface slime aggregating 30 or 40 cubic millimeters, entirely free from fragments of the substratum (which contained undestroyed starch), were shaken in a beaker with 150 c. c. of distilled water, and then put under an air pump for one-half hour, so as to remove air from the slime and permit penetration of the water into all parts. This water was then poured off, more added, and the exhaustion repeated. This second water was also poured off, more added, and the beaker again put under the air pump. After the third exhaustion there remained several hundred small bacterial fragments (zoogl&oelig;æ). As much as possible of the water standing over them was then poured off and 4 c. c. of iodine potassium iodide was poured into the beaker and allowed to act for 20 minutes, during which time all of the fragments became yellow. To this fluid was then added 4 c. c. of the c. p. sulphuric acid water (2:1). In an hour's time there was not the faintest trace of any blue reaction, all of the bacterial fragments remaining yellowish brown. Some unwashed masses of bacteria, carefully removed from the surface slime of one of these banana cultures, were now thrown into the beaker. Their surface immediately blued, and in a few minutes each one of these masses became deep blue throughout, forming a very striking contrast to the yellow stain in the washed particles.

It may be that substances absorbed from the substratum into the bacterial layer will account for all of the few recorded cases of cellulose reaction in the bacteria. This possible source of error is certainly worthy of very careful consideration.

VITALITY.

Length of Life in Culture Media.

No special attention was given to this subject, but from time to time, for various purposes, tubes of suitable culture media were inoculated from old cultures. The results show that *Ps. hyacinthi* is not readily destroyed by its own decomposition products. The nature and age of the old cultures in which this organism was still alive are given below: Feebly (litmus) alkaline potato broth, 24 days; beef broth neutral to phenolphthalein, with 5 per cent cane sugar, 32 days; moderately alkaline beef broth, with 10 per cent cane sugar, 79 days; acid (unneutralized) beef broth, 26 and 64 days; feebly (litmus) alkaline slant agar, 24 days; nutrient starch jelly, 31 days; sugar beet, 52 days; coconut, 59 days; white turnip, 41 and 80 days; radish, 80 days; gelatin neutral to phenolphthalien, 38 days; gelatin alkaline to phenolphthalein, i. e., −20 of Fuller's scale, 156 days; malic acid gelatin (acidity +54 of Fuller's scale), with 10 per cent cane sugar, at temperatures ranging from 10° to 25° C., 174 days. In a potato culture 91 days old the organism was dead. It was also dead after 33 days in a beef broth made feebly alkaline to litmus by sodium carbonate. It was dead in 5 coconut cultures at the end of 2 years; it was dead on sugar beet after 2 years and 10 months; it was dead in 3 cultures on agar (stock 527) after 17½ months. These results indicate that the organism is fairly resistant, and also that it produces very little organic acid.

Ps. campestris, *Bacillus amylovorus*, *B. carotovorus*, *B. pyocyaneus pericarditidis*, and a green fluorescent germ which grows in the presence of thymol, and which was isolated by the writer from one of Dr. Loew's tobacco infusions, were all alive on agar (stock 527) after 17½ months. *Ps. stewarti*, on the contrary, was dead (2 tubes). *Ps. phaseoli* was also dead. All of these tubes were in the stock-culture box, subject to the same degree of cold (temperature 5° to 16° C.), moisture, and darkness. Under similar conditions *Ps. campestris* was alive on potato after 5 months and on agar (stock 553) after 10 months. Several tubes of *Ps. phaseoli* were alive after 5 months on potato. *B. carotovorus* was also alive on potato at the end of 5 months.

Resistance to Dry Air.

Dr. Wakker states that the hyacinth organism remains alive in a dry state for a long time. Only three experiments were made to determine this point, all of which tend to confirm his statement.

(1) A typical potato culture 9 days old was shaken until nearly all of the yellow slime was washed into the 1 c. c. of fluid in the bottom of the tube. Fifteen small drops of this heavily clouded fluid was then spread on 15 small, clean, sterile cover glasses, in a Petri dish, the cover replaced, and the dish set away in a dry, dark closet, at 20° C., for 9 days. At the end of this time 13 of these covers were dropped into as many tubes of culture media—beef broth, sugared peptone water, etc.

Result: *Ps. hyacinthi* developed after a few days in all of these tubes, showing that some germs were still alive on each cover glass. The time required to cloud these tubes was 3 to 8 days, at 16° to 20° C.

(2) The remaining 2 covers were kept until the 47th day, after which they were put into 1 per cent grape sugar peptone water.

Result: After a few days the fluid in each tube clouded and threw down a yellow precipitate.

(3) Some weeks later this experiment was duplicated, with the exception that a period of 48 days was allowed to intervene between the spreading of the cloudy fluid on the covers and their submersion in the culture medium. In this instance the bacteria were derived from a 9-days-old culture on yellow banana, the slime being rubbed over the clean sterile covers, which were then set away as before. On the 48th day 18 of these covers were seized with sterile forceps and dropped into as many tubes of sterile beef broth (stock 382) and set away in the dark, at 20° to 26° C.

Result: All but one of these tubes developed *Ps. hyacinthi.* Nine clouded on the 4th day; 5 on the 8th day; 2 on the 12th day. Two tubes were clear on the 17th day, but one of these was cloudy on the 23d day. The other remained clear. These results seem to indicate a marked difference in the vitality of individual rods. These are the cultures which were tested for invertase.

Experiments with *Ps. campestris* and *Ps. phaseoli* show that they are also resistant to dry air, but apparently less so than *Ps. hyacinthi.* The organisms were dried on cover glasses in a dark closet in the same way as *Ps. hyacinthi*, except that the temperature averaged about 5° higher. The covers were inoculated copiously and were side by side in clean covered Petri dishes. Of course those inoculated from the potato received the most bacteria. The tests were made from solid and fluid cultures and into two kinds of beef bouillon, viz: (1) Stock 577, a standard salted peptonized beef broth +15 of Fuller's scale, which had dried out one-fourth by long standing; (2) stock 579, a flask of stock 577 diluted with an equal bulk of distilled water before it was filled into the test tubes. When everything was ready for the test, the dishes were brought out of the closet, and in clean, still air the inoculated covers were seized with sterile forceps and dropped one after another into the tubes of beef bouillon, which were then replugged, set away in the dark, and watched for six weeks.

These experiments were as follows:

(1) *Ps. campestris.*—Covers inoculated copiously from the yellow slime on a potato culture 2 days old. Dry 34 days.

a. Covers thrown into stock 577—12 tubes.

Result: One tube clouded on the 3d day and one on the 4th day. The other 10 remained clear. The clouding was typical for this organism, and cultures made from each of the tubes into potato yielded a typical growth of *Ps. campestris.*

b. Covers thrown into stock 579—12 tubes.

Result: Six tubes were cloudy on the 3d day; 6 remained clear. The clouding was typical, and cultures from each of the 6 tubes into tubes of potato yielded in each case pure cultures of *Ps. campestris.*

(2) *Ps. campestris.*—Each cover inoculated with a small drop of moderately cloudy fluid from a beef broth culture 2 days old. Dry 34 days.

a. Covers thrown into stock 577—11 tubes.

Result: No growth in any of the tubes.

b. Covers thrown into stock 579—12 tubes.

Result: Two tubes clouded on the 3d day. The rest remained clear. The clouding was typical, and transfers from the tubes into tubes of potato yielded pure cultures of *Ps. campestris.*

(3) *Ps. phaseoli.*—Covers inoculated copiously with the yellow slime from a potato culture 3 days old. Dry 27 days.

a. Covers thrown into stock 577—9 tubes.

Result: One tube clouded on the 3d day, one on the 5th day, and one on the 6th day. The rest remained free. The clouding was typical, and cultures from each tube into potato yielded a pure growth of *Ps. phaseoli.*

b. Covers thrown into stock 579—12 tubes.

Result: Two tubes clouded on the 3d day and 2 on the 4th day. The rest remained free. The clouding was typical, and cultures from each of the tubes into tubes of potato yielded a typical growth of *Ps. phaseoli.*

(4) *Ps. phaseoli.*—Each cover inoculated with a small drop from a well-clouded beef-broth culture 3 days old. Dry 27 days.

a. Covers thrown into stock 577—12 tubes.

Result: All clear to the end of the experiment.

b. Covers thrown into stock 579—10 tubes.

Result: No clouding in any of the tubes.

Conclusion: In each case the transfers from potato did better than those from beef broth. The dilute bouillon appeared to be a more favorable medium than the concentrated. *Ps. phaseoli* seems to be less resistant to dry air than *Ps. campestris.*

RESISTANCE TO SUNLIGHT.

The writer's experiments have not been very numerous, and the shortness of exposure absolutely necessary for the destruction of the organisms is not known, but probably it is considerably less than the time given below. The tests were made in poured plates of nutrient agar, which was inoculated copiously. The exposures were made in very thin-bottomed Petri dishes lying bottom up on larger Petri dishes filled with pounded ice. The exposures were made in Washington in May. One-half of each plate was covered by several folds of thick paper and the other half exposed to the unclouded sun. A good-sized drop of well-clouded fluid was used in making each inoculation, i. e., many thousands of the bacteria. The bacteria in the covered portion of the dishes developed normally (except near the margin of the paper covering) as a dense uniform sheet of crowded small colonies. On the exposed part of each plate, and for some millimeters beyond, nearly all of the bacteria were destroyed. The few that remained were tardy in development and undoubtedly owed their escape to the protecting shade of less fortunate individuals. The exposed plates were as follows:

1. *Ps. hyacinthi.*—30 minutes' exposure; all killed.
2. *Ps. hyacinthi.*—45 minutes' exposure; all killed.
3. *Ps. campestris.*—30 minutes' exposure; 95 per cent killed.
4. *Ps. campestris.*—45 minutes' exposure; 98 per cent killed.
5. *Ps. phaseoli.*—30 minutes' exposure; 98 per cent killed.
6. *Ps. phaseoli.*—45 minutes' exposure; all killed.

The exposure was at midday. The temperature of the plates during the experiment ranged from 24° to 27° C., i. e., was held down satisfactorily by the ice. In each case a considerable portion of the bacteria were killed under that part of the cover nearest to the exposed portion, i. e., over a width of one-fourth inch or more. On this part the colonies developed slowly at first, and, being fewer, had more room to grow, and became larger than on any other portion of the covered part of the plates. The covered part of each dish was turned south, i. e., toward the sun.

Stewart found that exposure of *Ps. stewarti* in a portion of an agar plate to bright sunlight for 3 hours destroyed nearly all of the organisms. In that part of the plate which was not exposed the yellow colonies came up thickly in 96 hours at 23° C.[1] He does not speak of having tried the result of shorter exposures. Russell and Harding found that exposure of *Ps. campestris* in agar plates for 15 minutes to an August sun (latitude 43°) destroyed 90 per cent of all the organisms. Similar cultures exposed for 30 minutes to a November sun remained entirely sterile.[2]

RESISTANCE TO HEAT.

Ps. hyacinthi is quite sensitive to heat, much more so than the bacterial parasites of the warm-blooded animals. To a less degree the same is true of *Ps. phaseoli* and *Ps. campestris*. See Temperature relations.

RESISTANCE TO ACIDS.

Ps. hyacinthi is quite sensitive to acids, being restrained from growth by comparatively small doses. It will tolerate more acid in a solid than in a fluid medium, and more of some acids than of others. See Malic acid gelatin and Sensitiveness to acids. In beef broth with malic acid +30 appears to be about its limit of growth.

RESISTANCE TO ALKALI.

Ps. hyacinthi will grow in −25 gelatin and in −20 beef broth, but experiments have not been numerous enough to determine just how much alkali it will endure. In gelatin and beef bouillon −30 of Fuller's scale is probably about the limit of toleration of caustic soda.

GROWTH IN PRESENCE OF CALCIUM SULPHITE.

Ps. campestris grew in 10 c. c. portions of galactose-peptone water with addition of 40 milligrams of calcium sulphite, but growth was distinctly retarded. The stock consisted of distilled water, ½ per cent peptone, and ½ per cent galactose. Other organisms were not tested.

GROWTH OVER CHLOROFORM.

This test was made by adding 5 c. c. portions of c. p. chloroform to 10 c. c. portions of sterile alkaline beef broth in cotton-plugged test tubes. The beef broth was stock 382,[3] well adapted to the growth of this organism. The chloroform settled at once to the bottom, but on unplugging its odor was always perceptible in the mouth of the tube.

---

[1] A Bacterial Disease of Sweet Corn. Bulletin 130. N. Y. Ag. Exp. Sta., Geneva, N. Y., 1897, p. 434.

[2] A Bacterial Rot of Cabbage and Allied Plants. Bulletin 65. Ag. Exp. Sta., Wisconsin. Madison, Wis., 1898, p. 19.

[3] 1,320 grams minced lean beef and 2,000 c. c. distilled water in ice chest 24 hours. Steamed, filtered, resteamed, added water to make fluid 2,640 c. c. Titrated and found +25. Added caustic soda to 0. A fermentation tube yielded 0.6 c. c. gas with *B. coli*. No peptone added.

The chloroform exerted a marked retarding influence on *Ps. hyacinthi*, but did not always prevent its growth. The tube was first inoculated with two 3 mm. loops from a 10-days-old moderately cloudy culture in Dunham's solution. In 24 days (at 20° to 25° C.) there was no growth. The tube was now reinoculated with two 3 mm. loops of cloudy broth from a 3-days-old culture in stock 382. After 12 days there was a faint surface clouding and a feeble partial rim of germs, which indicated that growth was proceeding with much difficulty. A month later there was a good, dense, yellow rim, 2 mm. wide, the fluid was well clouded, and on top of the chloroform there was a loose yellow bacterial precipitate about 2 mm. deep.

For comparison with *Ps. hyacinthi* tubes of the same medium were inoculated with other organisms. Under the same conditions *Ps. campestris* refused to grow. The tube was first inoculated with two 3 mm. loops from a 10-days-old moderately cloudy culture in Dunham's solution. After 24 days, there being no growth, the broth was reinoculated with two 3 mm. loops from a well-clouded 3-days-old culture in stock 382. After 43 days, there being no growth, the tube was inoculated for the third time with a 2 mm. loop of solid slime from a 48-hour growth on the surface of cooked turnip. This slime was broken up in the fluid by means of the platinum loop, and afterwards divided to a still greater extent by shaking the fluid thoroughly. The tube was under observation for an additional 13 days, but no growth ensued.

Ps. stewarti, on the contrary, grew in this chloroformed beef broth abundantly, with only slight retardation, and remained alive in it for more than 2 months. The tube was inoculated with one loop from a 10-days-old culture in Uschinsky's solution.

A number of other organisms behaved in much the same way as *Ps. stewarti*, i. e., they were more or less retarded for a few days, but afterwards made a more or less copious growth. Among these were *B. amylovorus*, *B. carotovorus*, *B. pyocyaneus pericarditidis*, and *B. coli*. *Ps. phaseoli* grew slowly in chloroformed cane sugar bouillon when inoculated copiously.

MEANS OF DISTINGUISHING THE FOUR SPECIES OF PSEUDOMONAS.

The four species of Pseudomonas may be distinguished as follows:

Found in ....

1. Cruciferous plants—cabbage, cauliflower, kohlrabi, kale, rape, turnips, rutabagas, mustards. *Ps. campestris.*
2. Leguminous plants—beans of various kinds, e. g., lima beans, bush beans. *Ps. phaseoli.*
3. Liliaceous plants—hyacinths. *Ps. hyacinthi.*
4. Gramineous plants—maize, especially sweet corn. *Ps. stewarti.*

Growth on steamed potato cylinders standing in distilled water.	1. Copious and prolonged, covering the potato and filling the water with a solid yellow slime and changing all of the starch within a few weeks so that it does not react blue or red with alcohol iodine or iodine potassium iodide. *Ps. campestris*, *Ps. phaseoli*. 2. Moderate and very little after the second week, not always covering all of the exposed part of the potato and never filling the water with a solid yellow slime, the starch but little acted upon and always yielding (even immediately under the slime) a pronounced blue, blue purple, or red purple reaction. *Ps. hyacinthi*, *Ps. stewarti*.
Growth in milk	1. The whey is slowly separated from the casein by means of a lab ferment; the casein slowly settles and after some weeks is partially redissolved. *Ps. campestris*, *Ps. phaseoli*, *Ps. hyacinthi*. 2. Growth good, but milk continues opaque and the whey never separates from the casein. *Ps. stewarti*.
Growth in litmus milk.	1. Blue litmus becomes gradually more and more alkaline. At no time is there any indication of acids. *Ps. campestris*, *Ps. phaseoli*, *Ps. hyacinthi*. 2. Blue litmus in course of some weeks changes to lilac or heliotrope, indicating the formation of a slight amount of acid. *Ps. stewarti*.
Growth on nutrient gelatin and Loeffler's blood serum.	1. A slow liquefaction, best in the order named. *Ps. phaseoli*, *Ps. campestris*, *Ps. hyacinthi*. The latter brightest yellow. 2. A good buff-yellow growth, but no liquefaction. *Ps. stewarti*.
Growth on steamed yellow turnips or rutabagas standing in distilled water.	1. Copious in the air and filling the fluid with a thick yellow slime, which is not iridescent; substratum browned and softened. *Ps. campestris*, *Ps. hyacinthi*. The latter Naples yellow, the former paler yellow. 2. Buff yellow, slightly iridescent, sparing (thin), and soon at an end, never filling the water with a solid yellow slime. Substratum not browned or softened. *Ps. stewarti*.
Growth in milk or bouillon with ethyl alcohol.	1. On boiling old cultures the steam yields an acid reaction and a fragrant smell. *Ps. hyacinthi*. 2. No such acid reaction or odor. *Ps. campestris*, *Ps. phaseoli*, *Ps. stewarti*.
Behavior in tomato juice and cabbage juice.	1. Did not grow. *Ps. campestris*, *Ps. phaseoli*, *Ps. hyacinthi*. 2. Grew copiously and for a long time without retardation (cabbage) or with only a slight retardation. *Ps. stewarti*.
Behavior in concentrated beef broth (acidity, −80).	1. No growth. *Ps. campestris*, *Ps. phaseoli*, *Ps. hyacinthi*. 2. Retardation for some days, then a copious and prolonged growth. *Ps. stewarti*.
Behavior in Dunham's solution with indigo carmine.	1. No immediate reduction; color slowly changes to a pure bright blue, which persists for several weeks, but finally fades through green to yellowish. *Ps. hyacinthi*. 2. No immediate reduction; color bluer for a few days only, changing to green and bleaching much sooner than the preceding. *Ps. campestris*, *Ps. stewarti*.
Behavior in Dunham's solution with methylene blue.	1. Marked reduction; on shaking, a prompt reoxidation (to blue); final color the same as at the beginning (pure blue); bacterial precipitate not stained. *Ps. hyacinthi*. 2. As above, but the final color of the fluid green. *Ps. campestris*. 3. No reduction, final color of the fluid blue; bacterial precipitate stained deep blue. *Ps. stewarti*.

Behavior in Dunham's solution with alcoholic solution of rosolic acid and a small amount of HCl.	1. Between the 6th and 9th day the pale orange yellow fluid changes to a geranium red, which gradually deepens to poppy red (37th to 56th day). *Ps. campestris.* 2. The color changes follow the same general course as in the preceding, but much more slowly; i. e., no distinct change of color until after the 16th day and not so deep on the 56th day. *Ps. stewarti.* 3. The yellow color of the fluid gradually bleached and practically all gone at the end of the second week; no reddening of the fluid. *Ps. hyacinthi.*
Behavior in chloroformed beef broth.	1. No growth. *Ps. campestris.* (Only one experiment.) 2. Slow, long-continued growth, but with much difficulty in getting started. *Ps. hyacinthi, Ps. phaseoli.* 3. Good growth, with little difficulty in getting started. *Ps. stewarti.*
Behavior in distilled water containing 4 per cent maltose and 4 per cent Witte's peptonum siccum.	1. Fluid in old cultures (40 to 60 days) distinctly browned. *Ps. campestris.* 2. Fluid not browned. *Ps. phaseoli.*
Growth on 10 c. c. slant nutrient agar containing 3 grams of cane sugar.	1. No distinct retardation, surface smooth, slime copious, and generally wet enough to flow readily. *Ps. campestris, Ps. phaseoli.* 2. Marked retardation of growth, surface roughened, reticulated or areolated, slime dry so that it does not flow. *Ps. hyacinthi.*
Behavior on 10 c. c. slant nutrient agar containing 1 gram of grape sugar.	1. Growth copious, stimulated from the start. *Ps. campestris, Ps. phaseoli, Ps. stewarti.* 2. Growth retarded for a week or more, but finally better than in the check tubes. *Ps. hyacinthi.*
Behavior on 10 c. c. of slant nutrient starch jelly containing 500 mgs. of glycerin.	1. Growth, after 24 days, copious, sirupy, bright yellow. *Ps. campestris.* 2. Growth, after 24 days, much less than in the preceding or than in the check tubes, and with no distinct yellow color. *Ps. phaseoli.*
Behavior on slant nutrient starch jelly made with modified Uchinsky's solution (see p. 63).	1. Growth good, slime yellow, marked diastasic action. *Ps. campestris.* 2. Growth much less abundant than in the preceding and slime very pale, marked diastasic action. *Ps. phaseoli.* 3. Growth feeble, no diastasic action. *Ps. hyacinthi.*
Behavior in Uchinsky's solution.	1. No growth or growth long delayed and feeble, with appearance in the fluid of small, whitish, loose, wooly flocks. *Ps. hyacinthi.* 2. Growth retarded and feeble, zoogloeæ compact, roundish. *Ps. campestris.* 3. As in 2, but a yellower and rather better growth. *Ps. phaseoli.* 4. An abundant and long-continued growth—a very suitable culture medium. *Ps. stewarti.*
Thermal death point (10 min. exposure in beef bouillon).	(1) 53° C. *Ps. stewarti.* (2) 51.5° C. *Ps. campestris.* (3) 49.5° C. *Ps. phaseoli.* (4) 47.5° C. *Ps. hyacinthi.*

Brightest color .....
1. Generally wax yellow. *Ps. campestris.*
2. Wax yellow to chrome. *Ps. phaseoli.*
3. Chrome yellow to canary. *Ps. hyacinthi.* The brightest yellow of the four.
4. Buff yellow to chrome. *Ps. stewarti.*

N. B.—Old cultures darken and stress must not be laid on slight differences in color at any age, since the yellow color of the same species varies according to the amount of brown pigment produced, and this varies with the medium and sometimes even with slight changes in the medium (see page 142).

REMARKS ON THE YELLOW PSEUDOMONAS GROUP.

Characters in Common.

These bacteria agree in the following particulars: They are yellow rod-shaped organisms of medium size, straight or slightly crooked, with rounded ends. The segments multiply by fission after elongation. They are generally less than 1 μ in diameter. The segments are of variable length. As taken from the plant or from ordinary culture media, they are seldom more than three times as long as broad, and are often much shorter. The segments occur singly, in pairs or fours joined end to end, or in clumpy masses of variable size (zooglœæ), more rarely they are united into long chains or into filaments in which no septa are visible. Endospores are absent or rare (none have been observed). The segments are motile by means of one polar flagellum, which is generally several times as long as the rod, and may be wavy or straight when stained. The species grow readily on all of the ordinary culture media, but so far as definitely known all require the presence of air—i. e., are strictly aerobic.[1] None are gas producers. All are sensitive to sunlight. All are quite resistant to dry air. They do not reduce nitrates to nitrites. As a rule, they are not easily destroyed by their own decomposition products. The yellow color appears to be a lipochrome. In the different species it varies from deep orange and buff-yellow, through pure chrome and canary-yellow, to primrose yellow and paler tints. In the same species the yellow color also varies somewhat, being frequently changed, darkened, or obscured by the production of a soluble brown pigment, the amount of which pigment varies in different species, and in the same species on different media. Organisms parasitic in plants or saprophytic.

As our knowledge increases it will, of course, be necessary to revise this characterization and probably to subdivide the group. *Ps. campestris* and *Ps. phaseoli* are nearly related; *Ps. hyacinthi* differs from the above very considerably, and *Ps. stewarti* is still further removed.

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[1] Note possible exceptions mentioned on pages 66, 67, and 71.

OTHER SPECIES BELONGING TO THIS GROUP.

The following species also belong to this group and appear to be distinct from the foregoing, but our knowledge of their cultural characters is more or less imperfect:

(1) *Ps. juglandis* Pierce. Parasitic on the young nuts, leaves, and stems of *Juglans regia* in California. The cause of an economically serious disease in walnuts. Resembles *Ps. campestris.* Pierce does not mention having attempted to inoculate his organism into cruciferous plants, but the writer has tried the reverse of this without success, viz, inoculations of *Ps. campestris* and *Ps. phaseoli* into young rapidly growing shoots of the walnut (*J. regia*).

(2) *Ps. vascularum* (Cobb). Parasitic on sugar cane in Australia and elsewhere. The vascular bundles are filled with a yellow slime, the canes are dwarfed, and the sugar content is reduced.

(3) *Ps. dianthi* (Arthur and Bolley). Isolated from carnations (Dianthus spp.), and supposed to be the cause of a spot disease. Common on the surface of carnation leaves, but now believed to be purely saprophytic.

(4) *Ps. amaranti* n. sp. Occurs on species of Amarantus (weeds in fields) in the Eastern United States, filling and browning the vascular bundles and hollowing out the tissues in their vicinity into closed cavities filled with this organism. The plants which are attacked are stunted, droop, and dry up without any visible cause. The organism is a short rod and when grown on culture media has more orange in its pigment than any others here described. On the whole, it seems to be most nearly related to *Ps. stewarti.*

(5) *Ps. malvacearum* n. sp. Parasitic on cotton (Gossypium spp.). This organism causes the very characteristic leaf disease known as Atkinson's angular leaf-spot, and also a water-soaked spreading spot-disease of the capsules comparable to that produced on walnuts by *Ps. juglandis* and on bean pods by *Ps. phaseoli.* This bacterium has nearly the same thermal death point as *Ps. campestris* and much resembles it in many other ways, but its slime is more translucent on potato, and it is not parasitic to cabbage. The writer has had this organism under observation for several years, and has successfully inoculated it into young cotton bolls and leaves. Tissues of the cotton plant which are not growing rapidly do not readily contract the disease. This yellow organism is not the same as the green fluorescent germ isolated by Stedman from rotting cotton capsules and named *Bacillus gossypina.* A full account of the cotton disease is in preparation.

BULLETIN No. 29. V. P. P.—83.

U. S. DEPARTMENT OF AGRICULTURE,
DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY.
ALBERT F. WOODS, Chief.

PLANT BREEDING.

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GARDEN.

BY

WILLET M. HAYS,
Professor of Agriculture, University of Minnesota.

1862 1889

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1901.

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---

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BULLETIN NO. 29. V. P. P.—83.

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1901.

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Division of Vegetable Physiology and Pathology,
Washington, D. C., January 28, 1901.

Sir: I have the honor to transmit herewith the manuscript of a paper on plant breeding, prepared by Prof. Willet M. Hays, of the University of Minnesota. Professor Hays has been engaged in plant breeding for a number of years and has done much to arouse an interest in the subject throughout the country. The Department of Agriculture, through its laboratory of plant breeding, several years ago actively took up the investigation of plant-breeding problems, and much work has been done in the improvement of cotton, corn, wheat, oranges, pears, grapes, etc. Results of great importance have already been obtained, and several papers treating of important factors of plant breeding have been published in bulletins of this Division and in the various Yearbooks of the Department of Agriculture since 1897. The present paper is of special interest to experiment station workers and others engaged in similar lines, and I respectfully recommend its publication as Bulletin No. 29 of this Division.

Respectfully,

Albert F. Woods,
Chief of Division.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

Page.

- Introduction 7
- General observations on plant breeding 10
  - Relation of plant breeding to wealth 10
  - Examples of results of breeding 13
    - The Wealthy Apple 13
    - The race horse, Messenger 13
    - Minnesota No. 169 wheat 14
  - The value of large numbers in breeding experiments 15
  - General facts concerning heredity 16
  - The use of variation illustrated 17
  - Records and score cards 24
    - Percentage score cards 25
    - Graphic score cards 27
- Methods of plant breeding 28
  - Relation of plant introduction to plant breeding 28
  - Choice of foundation stocks 30
  - Names and numbering of varieties 31
  - Breeding by selection 33
    - Selection of seedling plants 33
    - Bud selection 35
  - Breeding by hybridization and selection 37
    - Degree of relationship in crosses 38
    - Hybrids and crosses defined 40
    - Importance and methods of hybridizing 40
    - Breeding for special and new uses 41
    - Breeding nitrogen into field crops 42
- Illustrations of work in plant breeding 44
  - Breeding wheat 44
    - Improvement by selection alone 44
    - Wheat flowers 50
    - Formation of varieties by hybridizing 50
    - Experiments in wheat breeding 54
    - Hybridizing as a cause of variation in wheat 56
  - Breeding corn 57
    - Selecting seed corn 58
    - Hybridizing varieties of corn 60
  - Breeding timothy 61
  - Breeding potatoes 63
  - Breeding apples 64
    - How to hybridize apples 65
  - Breeding black walnuts 68
  - Breeding flax 69

ILLUSTRATIONS.

PLATES.

Page.
PLATE I. Fig. 1.—Testing the gluten of new wheats as to its amount and strength; Fig. 2.—Making the bakers' sponge test of new varieties of wheat 28
II. Fig. 1.—Improvement of flax by selection; Fig. 2.—Planting seeds in the crop nursery 34
III. Fig. 1.—Two centgener plats of wheat; Fig. 2.—Men emasculating and cross-pollinating wheat 46
IV. Fig. 1.—Harvesting twentieth-acre plats of wheat; Fig. 2.—Thrashing and cleaning wheat from centgener plats 48
V. Milling samples of wheat 50
VI. Fig. 1.—Spikes of parent and hybrid wheats; Fig. 2.—Spikes of timothy, showing improvements by selection 56

TEXT FIGURES.

FIG. 1. Graphic expression of Quetelet's law 18
2. Yields in grams of 80 Powers Fife wheat plants, arranged in order as numbered 18
3. Yields in grams of 80 Powers Fife wheat plants, arranged in order of yield 18
4. Yields in grams of 80 plants of Blue Stem wheat, arranged in order as numbered 19
5. Yields in grams of 80 plants of Blue Stem wheat, arranged in order of yield 19
6. Yields in grams of 86 Fife-Blue Stem hybrid wheat plants, arranged in order as numbered 19
7. Yields in grams of 86 hybrid wheat plants, arranged in order of yield 20
8. Yields in grams of 100 Blue Stem and 100 Fife wheat plants compared 0
9. Yields in grams of 100 plants each of Fife, Blue Stem, and a hybrid between the two 21
10. Yields in grams of Fife, Blue Stem, and hybrid plants after further selection 22
11. Yields in bushels of 10 new strains of wheat 23
12. Graphic score card comparing wheats 26
13. Scheme of planting wheat in field crop nursery 45
14. Method of preserving record sheets 49
15. The spikes, flowers, and seeds of wheat 51
16. Opening of wheat flowers and anthers 52
17. Removing the flowers of the smaller and later spikelets 53
18. Opening the floret to remove the anthers 53
19. Graphic expression of the results of an experiment 57
20. Floral organs of timothy 62
21. Flowers of flax 70

PLANT BREEDING.

INTRODUCTION.

Much scientific thought has been centered on fertilizing the soil that the largest possible returns might be secured from a given amount of labor and expense. Man has been slow to recognize that plant life is in a wonderful manner subject to his control. There has been a controlling philosophy connected with botanical thought which has held too closely to the doctrine of the immutability of species in plants. The systematic botanist held the species so close to our eyes that the mobile character of the plants was not generally recognized. That "like begets like" is an important fact; but it is of greatest importance when used to intensify or fix variations of peculiar merit that these variations may be multiplied and thus become the basis of improved varieties. The results of breeding and the science of breeding have come forward slowly and unobtrusively. Their significance has not been fully appreciated. In view of the great results from breeding which have already appeared, it is safe to assume that persistent systematic effort will bring improvements that are now generally deemed impossible.

In any country there are very many localities each with its special conditions and needs. Each locality uses not a few, but many, species and varieties of plants, each suited to the particular soil, climate, and other conditions. Now that the creative work of plant breeding has been taken up by many scientific workers in State experiment stations, the national Department of Agriculture, and by many seed firms and individuals, variety formation and scientific breeding are bound to develop very rapidly. The suggestive work of Charles Darwin is at last vivifying the dormant ideas concerning breed and variety formation. Weismann and others who study heredity will continue to be heard with interest; and those who are studying methods of plant breeding to increase production will be heard in proportion to the economic and artistic value of their products and to the scientific value of their theoretical discoveries. Many of the facts relating to heredity are not only of great interest but of great value to the breeder. The study of the cell and of embryology is adding knowledge of exceedingly great value to the breeder. The known facts of animal and plant breeding, largely wrought out in extensive practice, and the visible results of the work of those who breed plants and animals, are for the present of paramount economic importance. They give the basis for a most valuable philosophy, because simple and useful. Plant breeding in America is very much underdone, and in many of its lines public money invested in experimental work may be made to yield to the country a hundred or a thousandfold. The literature of the subject of plant and animal breeding has not been as hopeful in tone as the achievements and the great importance of the subject have warranted.

The knowledge of the subject of breeding has not been well classified, nor has it been properly emphasized. There are a few books, many scattered articles, and numerous statements in the general writings of many men. Charles Darwin, in his work on "Variations of animals and plants under domestication," and other writings, brought together a large collection of facts and a most original philosophy of heredity and variation. He recognizes the force of facts shown by improvements which men had achieved in breeding plants and animals. J. H. Wallace, in Volume II of Wallace's American Trotting Register, and in other writings; J. H. Sanders, in his book on Horse Breeding; Manly Miles, in Stock Breeding; and other American writers on animal breeding, have shown that they in part comprehend the force of Darwin's philosophy as relates to animal and plant breeding. Prof. L. H. Bailey and other recent writers have done much to arouse an interest in breeding plants. The plant improvements of most marked prominence are recent, and the men who have done the best work have written but little. They have worked with plants rather than with the written theories. The theory of many writers gives less encouragement for far-reaching results than the facts warrant. The long time required to produce considerable changes in the species and varieties of useful plants has had a very retarding effect upon progress in this line of work. Results of immense economic importance which have already been attained should be shown in their true light. Where the individual can not afford to wait for results and will therefore not properly carry forward variety formation, it is the duty of the State to assume the work. The length of time required to breed special varieties of each class of economic plants suited to each locality should not, henceforth, prevent the expending of much effort in this direction.

Besides being a subject of vast economic importance, the breeding of plants is a fascinating study and a most enchanting pastime. It is one of those subjects in which students like to specialize. Students in the writer's college classes, as soon as they gain a place to work in the field-crop nursery and seed house, wish to forego specializing in other subjects and concentrate their energies on this fascinating and productive work. In looking toward the future, it seems probable that plant breeding will receive far more attention than heretofore. In European countries much more attention is given to the improvement of plants than in America. In two towns in Germany there are nearly 30 large seed firms, with an aggregate capital investment probably five times as great as the capital of the general seed firms of America. In that country owners of large farms breed and raise seeds of wheat, oats, rye, sugar beets, potatoes, and other crops. They get better profits from this work, and they build on their farms chemical and seed laboratories where the seed selection is done systematically. Those farmers to whom they supply seed which will make their yields larger are also benefited.

In England, the average yield of wheat is over 30 bushels per acre, while in the United States it is below 15 bushels. This difference is in part due to the better preparation of soil and the moister climate; but doubtless it is also due in part to the selection and breeding of wheat during a much longer period than has passed since wheat was first grown in this new country.

Dr. Rimpau, Mr. Haine, Mr. Strube, and other farmers of Germany, which country I had the pleasure of visiting recently, find that seed growing and breeding is a very practical vocation. They breed wheat in a manner somewhat similar to the plan described in the following pages, and they employ system and precision in the selection of individual plants and of resulting varieties. Mr. L. H. Haynes, of Fargo, N. Dak., has done much careful work in breeding wheat, and has made greater profits from his farm than if he had raised the standard varieties of wheat for the market.

Breeding plants, since the literature on the subject is growing in volume, gives opportunity for wide study. Especially as an art does the breeding of plants give the opportunity for the development of skill of a high order. The breeders of plants, as well as the breeders of animals, need to make more of a study of records, of relationships: in a word, design, collect, and study pedigrees of the plants and animals with which they deal.

In many cases amateurs who have followed up accidental forms of value have sold them to dealers at good prices, and some amateurs breed plants according to a carefully considered system, making a profit for themselves and at the same time producing valuable varieties for cultivation. Bright young farmers can not undertake work which is better calculated to add interest to their chosen business than to become breeders of pedigree plants or animals. Seedsmen depend largely upon amateurs for new and valuable varieties, and they are always ready to pay good prices for useful new sorts. The seedsmen of America have not kept pace with European seed firms in variety formation, nor even in keeping up and improving old forms. They have here a most legitimate field; unless they develop this feature, they remain only seed merchants, and can hardly hope to gain that position in the minds of purchasers which they should hold to have the most profitable seed trade.

Plant breeders need not work along narrow lines nor follow set rules. There are many ways of conforming to the broadly applicable principles of producing, finding or discerning, fixing, and multiplying desirable variations. The devices of each plant breeder will solve the problem for his environment and for the objects he seeks. Plans outlined to suit the needs of one may not wholly meet the requirements of another situated differently.

The great system of American experiment stations has fairly begun the work of variety formation in important plants. We can, therefore, hope for the subject to be gradually placed upon a thoroughly scientific basis. There is room and need in this line of work for the best energies of all the agencies, amateurs, seed growers, seed firms, and experiment stations, including that largest one, the national Department of Agriculture.

The writer is under obligation to Mr. Andrew Boss, assistant in agriculture in the University of Minnesota, and to others who have assisted in the experiments (as yet not all reported in station bulletins) upon which this paper is in part based; also to student assistants who have aided in preparing the illustrations.

GENERAL OBSERVATIONS ON PLANT BREEDING.

RELATION OF PLANT BREEDING TO WEALTH.

The economic results from plant improvement are already enormous in the aggregate, and the possibilities for the future are so great as to be truly dazzling. Circular No. 11, recently issued by the Division of Statistics of the U. S. Department of Agriculture, for example, estimates the world's wheat product for 1899 at over 2,500,000,000 bushels. Assuming the yield to be 20 bushels per acre, this required 125,000,000 acres of land. In ten years the Minnesota station, by careful breeding, produced a new variety of wheat, which yielded nearly 25 per cent more grain on the university farm than its parent variety, which was the best variety generally grown in the State. The following table (Bulletin No. 62, Minnesota State station) shows comparative yields of the new and the parent variety on several experimental farms. In the last two columns appear the average results obtained in five successive seasons at the university farm, near Minneapolis. It should be observed that this new variety has not proven so much superior in yield to the parent variety on other experimental farms a few hundred miles distant, which emphasizes the need of breeding varieties for each locality:

Minnesota No. 169 compared with its parent variety, Minnesota No. 51.

Where grown.	Season.	Yields per acre.		Yields per acre at university farm.	
		Parent variety.	New variety.	Parent variety.	New variety.
		Bushels.	*Bushels.*	*Bushels.*	*Bushels.*
University farm	1895	21.6	37.8	21.6	37.8
Do	1896	24.6	25.0	24.6	25.0
Do	1897	20.4	24.3	20.4	24.3
Do	1898	23.3	26.3	23.3	26.3
Do	1899	25.9	28.8	25.9	28.8
Northeast farm	1898	23.0	19.3		
Do	1899	11.7	13.4		
North Dakota	1898	33.5	38.4		
South Dakota	1898	20.2	14.1		
Iowa	1898	8.8	12.5		
Average		21.3	24.0	23.2	28.5
Gain			2.7		5.3

Twenty-five per cent, or 5 bushels per acre, increase would add to the world's supply of wheat 625,000,000 bushels. This, at 80 cents per bushel, would be valued at $500,000,000 for each year. If by breeding we can increase yields only so much as 5 per cent, or 1 bushel per acre, we will still have an increase of $100,000,000 per annum, or 1,250,000,000 bushels increase in the world's crop in ten years. Since the United States produces about one-fifth of the world's wheat supply, an annual increase of 1 bushel per acre would result in ten years in an increased valuation of $200,000,000 for this country. One-tenth of 1 per cent of this sum spent in wheat breeding would doubtless be more than sufficient to produce a much greater increase than 1 bushel per acre. The writer's interpretation of the facts concerning the production of numerous new varieties of wheat in Minnesota by selection, aided by occasional hybridizing, is that 25 per cent increase in yield is a conservative estimate of what it is practicable ultimately to accomplish in that State in the improvement of wheat by breeding. Several decades will be required to accomplish this, and the proof is now nearly conclusive that breeding, or at least variety testing, must be done for each general locality within the State.

The breeding of corn in the United States has resulted in a very marked improvement of that crop. In nearly every county there are varieties or strains specially suited to local conditions. Formerly those dent varieties which yielded large crops of grain were adapted only to the Southern and Middle States. Now dent varieties are found maturing large crops nearly to the center of the northernmost tier of States. Through selection the farmers of the Northern States have greatly increased the yields of their cornfields. Corn being such a large plant that each ear is taken in the hand in husking out the crop, the farmer has been able to choose the very best yielding plants out of immense fields. Without fully recognizing the fact, the farmers of America have been conducting with corn the most extensive breeding experiment ever carried on.

Corn being an open fertilized species, large numbers of new forms have resulted from accidental hybrids between different varieties in adjacent fields, thus forming many local varieties of this very interesting plant, and building up the yield and quality of the corn crop of America.

If the corn crop of the United States were reduced to 80 per cent of its present yield we would have 1,600,000,000 instead of 2,000,000,000 bushels, or an average of 20 instead of 25 bushels per acre. What farmer would think of returning to the smaller ears of fifty or one hundred years ago? Yet it is quite possible in another half century, by more careful breeding and with greater attention to the composition and quality of our corn, to make as important improvements as have been made in the past. It is probably safe to say that the farmers, by adding 25 per cent more to the care and labor of rotating crops, manuring the soil, and cultivating the corn, could secure 25 per cent larger yields. And it is probably just as safe to say that if one-tenth of 1 per cent of the corn crop's value were devoted by the Government to breeding this plant so as to better adapt it to each locality, 10 per cent more could be added to the yield. While better farming and better cultivation are ultimately the more important in the aggregate, plant breeding is relatively more important until our crops are brought up more nearly to their possible maximum of yield.

Our ten leading field crops in the United States yield an annual income which is valued on the farm at something like $2,000,000,000. No man who has earnestly and intelligently tried to increase the yields of any one of these crops will doubt the assertion that by breeding alone, other conditions remaining the same, an average increase of 5 per cent could be added to the yields of these ten crops in twenty years by a line of thorough experimentation. Prices remaining the same, this would add $100,000,000 annually to the aggregate valuation of these crops; or, in twenty succeeding years, $2,000,000,000. If to the increase in value of our principal field crops are added the increase in values of orchard, garden, greenhouse, and forest crops, we will have a much greater aggregate gain. All these crops are capable of improvement by breeding, the same as corn and wheat, and the general principles to be followed are the same throughout. In many of the flower and vegetable crops changes have already been produced by breeding that are far greater than the anticipated changes in the yields of corn and wheat.

In the case of sugar beets, for example, the percentage of sugar in the juice of the roots has been increased probably 100 per cent by rigid scientific methods practiced on a large and expensive scale by

European seed growers. This work, started by Vilmorin of France, has made possible a large industry, profitable to the farmers and to manufacturers, and has resulted in much cheaper sugar for the entire world. Here, as in other lines of breeding, the principles and practice are comparatively simple and easily mastered. Remove it from the domain of abstruse reasoning, where some teachers of heredity place it, and plant improvement becomes a practical business proposition, an important affair of state.

EXAMPLES OF RESULTS OF BREEDING.

Three illustrations from plant breeding and from animal breeding, in which the general principles are the same, will suffice to emphasize the simpler side of the question of improving our useful plants:

THE WEALTHY APPLE.

The Wealthy apple, originated by Peter M. Gideon, of Minnesota, will serve as the first illustration. Mr. Gideon planted many apple seeds and watched the seedling trees develop. Most of the young plants succumbed to the severe Minnesota winters. One plant stood out prominently as being very hardy, and as the years passed it grew to a fruitful tree. Its fruit was fine in appearance and of superior quality. Mr. Gideon grafted some of the scions on other trees, and others he grafted on seedling roots, making independent trees. True to the nature of the apple tree, all these cuttings grew and bore fruit like that of the seedling plant. Mr. Gideon gave trees to his horticultural friends, and, being a nurseryman, sold many to his customers. This variety of apple now stands as a testimonial to Mr. Gideon's usefulness. He since has added a number of other useful hardy varieties to the apple list of the middle Northwest. The Wealthy apple, being the first prominent product of the efforts at breeding a hardy race of apples for the section of country mentioned, has a peculiar interest. As is often asserted, this apple, considered merely as a fruit product, may be worth more than a million dollars, but its value as an encouragement to apple breeding, and to plant breeding generally, is far greater. There are now being bred in Minnesota and surrounding States very many new varieties of apples from hardy parents.

THE RACE HORSE, MESSENGER.

Messenger, an imported English race horse, which became the leading progenitor of the American race of trotting horses, will serve as the second illustration. In nearly if not quite all of the best individuals of this great breed there is some of the blood of this horse, famous, not for his individual performance, but because of his power to transmit to so great a progeny the ability to win trotting races. This ability to trot is made up of many correlated elements, such as the instinct to contest the race, the form of body which permits light and free action, the texture of every bone, fiber, and nerve, and the teachable nature which enables the trainer to educate the horse. A combination of all these characteristics was given to this race of horses by the parent horse. Many other running horses have been employed in efforts to produce a competing strain of trotters. But the blood of Messenger stands above them all. And his descendants, through rigid selection extensively practiced, are gaining in trotting ability from year to year. He was a chance discovery. With the element of variation once in hand, the horsemen of America have gone on improving and intensifying it and reducing the American trotters to a uniformly fast-trotting race of animals.

MINNESOTA NO. 169 WHEAT.

One of the varieties of wheat originated by the Minnesota experiment station will serve as the third example. This wheat, mentioned on a previous page, was originated from a single plant (No. 476, of 1892) in the following manner: Several of the best plants were chosen from among 400 plants of Blue Stem, each growing separately, a foot apart each way. Each of the selected plants yielded 500 or more grains of wheat weighing 10 or more grams. The seeds from each chosen plant were planted for a few years until sufficient seed was obtained to plant a field plot. Then for several years each of the new strains was grown in a field beside the parent variety from which the 400 original seeds were chosen. A few of the new strains proved superior to the parent variety, but the one named Minnesota No. 169 stood out so preeminently superior that all others were discarded. For a large area of Minnesota this wheat seems capable of yielding at least 1 or 2 bushels per acre more grain than its parent variety, which is the best kind commonly and almost universally found on the farms in southern and central Minnesota. This variety in ten years could be increased so as to almost displace the parent wheat. The peculiar quality or power in the single germ which was the parent of plant No. 476 in our field crop nursery in 1892 had a very great value. The system which was followed to find the germ of greatest value is comparatively simple. The cost of finding this plant and of developing a variety of wheat from it, including the cost of forming and testing all the strains which failed to reach first place, was probably not one-thousandth of the value of this new wheat. The production of this and other similarly useful new varieties gives courage to experimenters, and warrants the State in investing more money in similar experimentation with wheat and other important crops.

The purpose of this paper is to show the importance of plant breeding to the country and to the farmers and gardeners, and to throw out into clear light its important practical features. The great question is, How can we get results? If a single germ has such wonderfully far-reaching power and immense value, as in the instances mentioned above, we want to know two important things: (1) How shall germs with the special values be produced? (2) How shall we select them out of the large number which must be grown to secure the superior one?

THE VALUE OF LARGE NUMBERS IN BREEDING EXPERIMENTS.

The history of breeding animals and plants has many lessons, only a portion of which are as yet read understandingly. The English breeder of hounds expressed a most important truth when answering an inquiry as to the secret of his success. He said: "I breed many and I hang many." Only the very few of highest value out of many were used as the foundation stock for further breeding. This does not mean that he did not keep many hounds in his kennels at one time. In most cases he could not determine the value of the blood of a certain sire or dam until he had numbers of his or her progeny. Not infrequently the blood of a certain animal or plant is unappreciated until, after its death, successive generations have shown the peculiar power of its "blood" to transmit valued characteristics. Messenger is appreciated more by horsemen every year. The peculiar value of the Blue Stem wheat plant, No. 476 of 1892, was not generally known until the several strains of wheat from the various wheat plants had been grown in the field trials for some years, and until the one springing from this plant had outstripped all the others as a valuable variety for practical planting in several States. Peter M. Gideon dug up many thousands of apple seedlings, and yet he is regarded as peculiarly fortunate in securing such a large proportion of valuable apple varieties from his extensive plantations of seedlings.

Luther Burbank, of California, in his work in producing new forms of valuable fruits and flowers, digs up and throws away annually acres of plants in his endeavors to find the one plant in many thousands from which he can produce a new variety with higher economic value or artistic qualities. The Garton Brothers, of England, in producing their new forms of cereal, forage, and root crops, have dealt with very large numbers of individual plants. The breeders of sugar beets analyze annually millions of sugar-beet roots, one firm alone employing 200 people in this work for two months each year, that the "blood" of the comparatively few best mother plants may be incorporated into new strains and varieties.

Cruikshank, the originator of the famous family of Shorthorn cattle known by his name, annually had under his eye very many of the finest Shorthorns of the British Islands, and with wonderful skill brought into his barns those very best animals which would most effectively center the blood of the best meat-producing quality of the whole breed into one herd. His philosophy, his artistic skill, his business ability, his far-seeing patience, and his long life resulted in his forming a subbreed of meaty Shorthorns which has made him famous and has produced great wealth for growers of cattle and users of meat.

Utilizing large numbers of the larger domestic animals, or such plants as forest trees and even some large fruit trees, is often so expensive as to be almost prohibitory. On the other hand, the breeder of wheat, corn, asters, or geraniums can inexpensively use large numbers and make correspondingly rapid progress. The principal part of the work in breeding is in eliminating the many poor ones, and plans for doing this effectively and cheaply without danger of discarding the few desired plants are very necessary. It is but natural that many of these broader laws or principles of business practice in plant breeding are being worked out by persons who are dealing with varieties and breeds with which it is practicable to employ very large numbers. Wheat, in addition to the advantage of using immense numbers at small cost, has many other characteristics valuable to the student of breeding and heredity. The "performance record" of each individual can be measured in a number of its important characteristics—as yield in weight of grain, quality of grain, size of kernels, height of plant, etc.—and these values may be expressed in numbers, so as to be averaged for a series of plants in one year or for a series of years. The seeds from each plant being numerous, a small plot can be planted from each of several mother plants, and by securing their averages of yield, quality of grain, height, etc., the transmitting powers of the respective parents may be easily compared. Field varieties may be developed from each of the several best stocks, and these, at a reasonable expense, may be tested in field trials as to yield and also as to their milling properties. Moreover, the seeds may be preserved for a number of years, so that the original variety may be grown and compared with progeny which has been bred for a series of years. The hoped-for benefits from extensive experiments in plant breeding being so great, it would seem that those interested in plant production and those concerned in the country's welfare would no longer be content until this work is placed on that adequate scientific and financial footing which its requirements and its importance demand.

GENERAL FACTS CONCERNING HEREDITY.

Many important facts concerning heredity and variation in their relation to plant and animal improvement are easily understood. There are innumerable facts and intricate theories regarding the cell and its contents, concerning fecundation, the development of the embryo, the growth of the individual and its life and death, which interest and fascinate the student. These interesting and highly important facts are so numerous that they may entice the would-be improver of plants to continue a student and not a practical producer of values. On the other hand, those who seek for values are too much open to the accusation of not properly reading, and of not recording for others the way nature would have the breeder follow in producing new forms. Both classes of men are needed; also those who broadly combine the scientific and practical.

Some of the important principles and facts to be observed in improving plants may be enumerated as follows:

(1) The individual plant produced from a seed is the important unit in plant breeding. The "bud unit," though of much consequence in case of marked bud variation, is usually of minor importance.

(2) Heredity, centripetal-like, enables us to produce from certain choice plants many descendants which, on the average, quite resemble their parents.

(3) Variation, centrifugal-like, causes the production among the descendants, along with very many average plants, of a few very good individuals and a few very poor ones.

(4) By selecting those best plants which upon trial produce superior progeny, the whole variety may be slightly or considerably improved.

(5) Since the plants of each succeeding generation also vary, by repeatedly choosing the best the variety or race is further improved.

(6) In many cases crossing increases the average vigor of the progeny, but in other cases it decreases the average vigor, size, or other desirable characteristics.

(7) In all cases crossing increases variation, as a rule, both toward better plants and toward poorer ones, thus giving opportunity for selecting from among the best plants individuals which are superior, as progenitors of varieties, to any individuals which could have been secured without crossing.

(8) New varieties can best be founded upon one to a dozen superior selected or cross-bred seedling plants used as parents.

(9) Very large numbers of individuals must be used from which to select or breed in order that mother plants may certainly be discovered from which superior varieties will spring.

(10) In addition to growing large numbers, the breeder of plants should grow all the plants of a given stock under uniform conditions, that they may be accurately compared.

(11) The testing of the finished variety must include adaptability to the soil and climatic conditions, the quality and value of the resulting crop, and the relative cheapness and practicability of its production.

THE USE OF VARIATION ILLUSTRATED.

Variation occurs in each and every characteristic of every class of living organisms. While nearly all of the individuals of a species, variety, strain, breed, or family resemble the average of their class in any chosen characteristic, there are a few which excel, and also a few which are considerably below, the medium in that quality.

This has been illustrated by Quetelet in taking the heights of many men chosen from the same race and reared in the same country. If a thousand men are arranged in a row in order of height, it will be found (1) that the man in the middle represents the average height of all the men; (2) that a line drawn over their heads will deviate from the horizontal but slightly throughout nearly its entire length, falling slightly toward the end where the shorter men stand; (3) that near the upper end the line will rapidly curve upward, and that near the lower end the line will rapidly curve downward. Figure 1 is the graphic expression of these facts. The men range from four feet and a fraction to nearly seven feet in height, the average being about $5\frac{2}{3}$ feet.

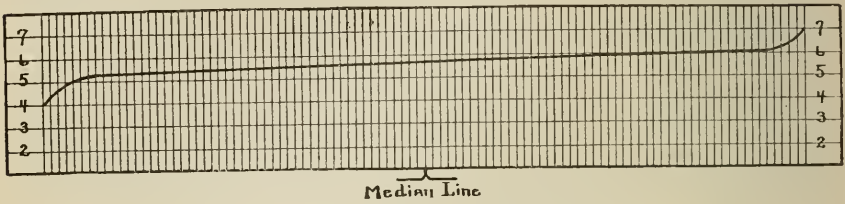

Fig. 1.—Graphic expression of Quetelet's law.

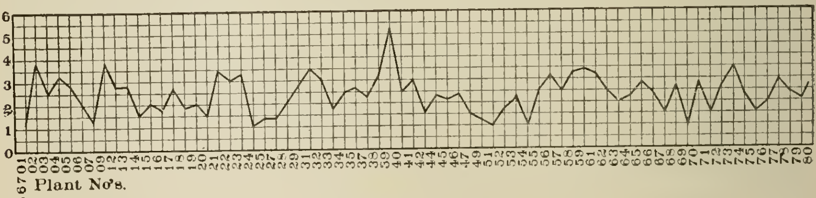

Fig. 2.—Yields, in grams, of 80 Power's Fife wheat plants grown in field-crop nursery, arranged in order as numbered. Each perpendicular line represents an individual plant, and the yield in grams is indicated by the horizontal lines numbered on the left, the length of line below the zigzag line representing the yield.

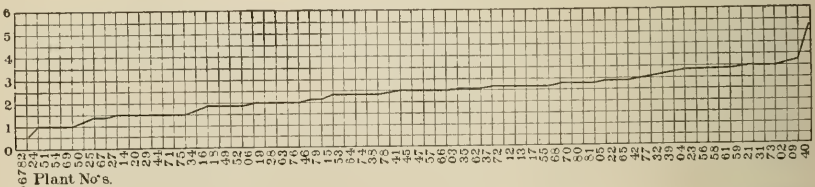

Fig. 3.—Yields, in grams, of 80 Power's Fife plants (the same as shown in fig. 2), arranged in order of yield.

The writer has applied this form of graphic expression to characteristics of wheat and other plants where the measures of qualities may be expressed in numbers, which in turn may be represented by the heights of vertical lines. In figure 2 is shown the yields of 80 individual plants of a new Fife wheat, which had been closely selected to type for some years, as they stood consecutively in a nursery centgener where each plant had 16 square inches of room. The vertical lines to the point where they are intersected by the curve represent the yields in grams, as shown by the figures standing at the left of the horizontal lines, the distances between which represent half a gram.

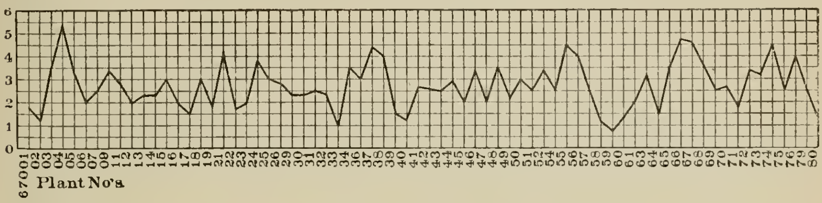

Fig. 4.—Yields, in grams, of 80 plants of Blue Stem wheat, arranged in order as numbered.

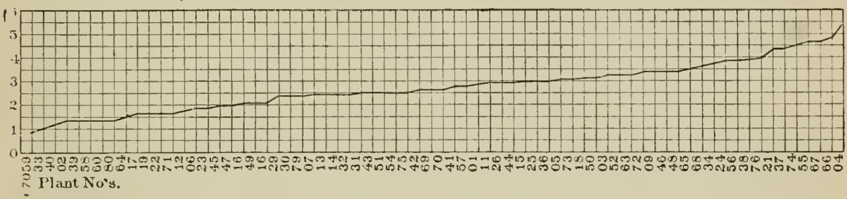

Fig. 5.—Yields, in grams, of 80 Blue Stem wheat plants (the same as shown in fig. 4), arranged in the order of yield.

In figure 3 these plants have been arranged in the order of their yield, displaying the curves. In figures 4 and 5, in like manner, are shown the yields of 80 Blue Stem plants, also long bred to a uniform type. In figures 6 and 7, in like manner, are shown the yields of 86 plants of a recent hybrid between Fife and Blue Stem wheats. It will be observed that there is much greater variation among the plants in the hybrid variety than among the plants of either parent variety. Accidents happen to many wheat plants, even in the carefully arranged

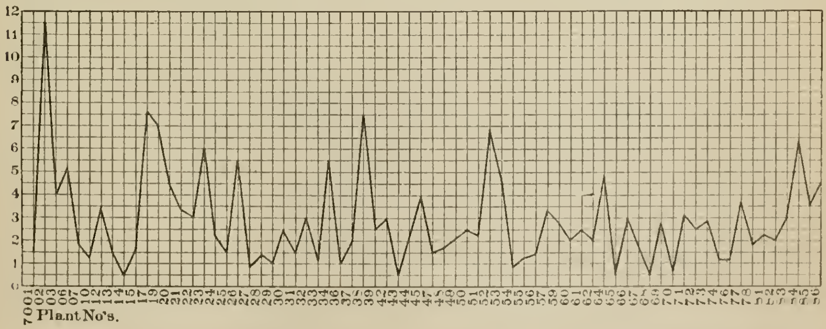

Fig. 6.—Yields, in grams, of 86 Fife-Blue Stem hybrid wheat plants, arranged in order as numbered, much greater variation being shown than in the parent varieties illustrated in figs. 2 and 4.

nursery; there is an abnormally large "infant mortality" and a large number of poorly yielding individuals, as shown in these curves.

This graphic expression is shown in its application to wheat selection in the more formal or theoretical curves in figures 8, 9, 10, and 11, based, only in a general way, on results of experiments in the field-crop nurseries and field-plot tests at the Minnesota experiment station. In figure 8 the solid-line curve cuts the vertical lines so as to represent the individual plant yields of 100 plants of Fife wheat, and the dotted-line curve does the same for 100 Blue Stem plants, arranged from left to right in the order of their yield in grams. The double horizontal line represents the mean between the field yields of these two varieties, as averaged from numerous field trials, and approximately the mean between the average yields of the plants of these two varieties in the field-crop nursery, where each plant has an area of 16 square inches, one plant in a hill, with a 4 by 4-inch space allotted to it. Since two standards of yield are used in these graphic expressions, an explanation is needed. Varieties of wheat yielding 20 to 30 bushels per acre in ordinary field-plot culture were planted also in the field-crop nursery plots, where the individual plants, standing singly in hills 4 by 4 inches apart and cultivated, yielded on an aver-

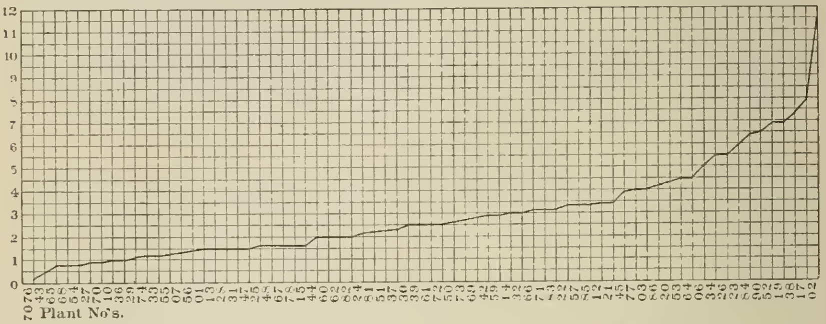

Fig. 7.—Yields, in grams, of 86 hybrid wheat plants (the same as shown in fig. 6), arranged in order of yield.

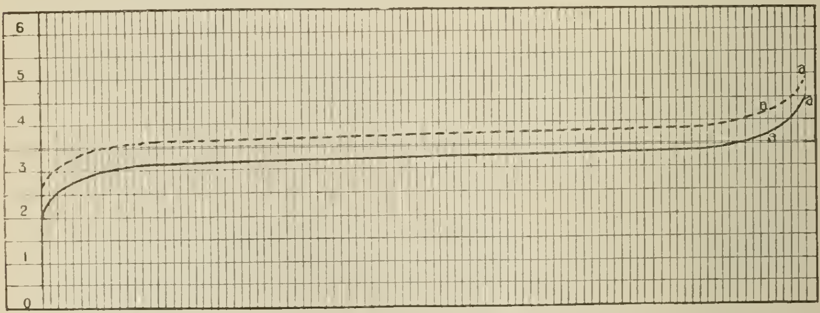

Fig. 8.- Yields, in grams, of 100 Blue Stem and 100 Fife wheat plants compared, the broken line representing the former and the solid line the latter. The mean yield is indicated by the double horizontal line.

age 3 to 4 grams per plant. Calculated in yield per acre, 3 grams per plant make a much greater yield than that of the ordinary field plot. In reading these graphic statements, based on general facts, this difference must be recognized in passing from the yields in grams of the plants in the plant-breeding nursery to the yields in bushels per acre of varieties grown in field plots.

The double horizontal line, at 23 bushels per acre, serves to indicate the standard average yield of the varieties used as a basis from which superior varieties of hard spring wheats rise under the breeding experiments. Since Fife wheat yielded 22 bushels per acre, as averaged for several years in the first field trials, and Blue Stem 24 bushels, the mean is taken as 23 bushels. In improving the yield of either of these wheats those plants are chosen whose vertical-yield lines rank them in the upper curve, as at *o—o*, plants which vary toward a better yield.

In figure 9 is shown, in addition to the Fife and Blue Stem curves of figure 8, a curve marked throughout by *x*, representing a hybrid between the Fife and Blue Stem wheats. This curve has more slant throughout its central straighter portion, and its upward curve goes higher, while the curve at the other end goes lower. This formal diagram has been so made that the center or average line is the same height as the mean between the parents of the hybrid. The mean here has little significance, since we do not expect to form a variety by using all of the hybrid progeny, but by using only the exceptional plants which rise into the upper curve.

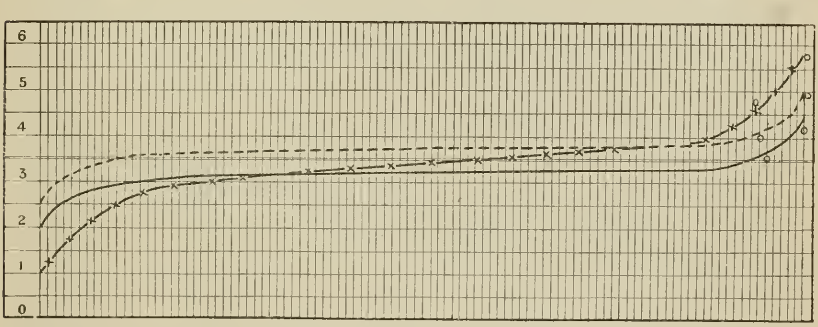

FIG. 9.—Yields, in grams, of 100 plants of Fife, Blue Stem, and a hybrid between the two. The yield of the Blue Stem is indicated by the broken line, that of the Fife by the solid line, and that of the hybrid by the line marked —*x*—. The best plants which should be used in further experiments are found near the end of the upper curves at the right and are indicated by *o—o*.

The important features in the hybrid are greater number of individuals from among which superior plants may be selected in the upper curve, and their large yields. These are more promising plants to use as mother plants in seeking varieties with better yields than are any of the plants ranking in the upper curves in the parent varieties. There has been created a power for greater yield. A "reaction" has been produced and the constancy of habit of the Fife and Blue Stem wheats to yield only 22 and 24 bushels, respectively, has been broken. Through the mixture of "blood" the centrifugal force has found a new place to center its efforts against the centripetal or conservative forces of heredity, and we have a few individuals varying in the desired direction of larger yield.

Variations thus newly created in seminally propagated varieties are generally not stable. The force of heredity of the family, race, and species powerfully combat the new characteristics and tend to reduce the new forms to conformity with the old. Most of the plants in the upper curve are very uncertain in their ability to transmit to their progeny their new and superior qualities, and those few which have this quality of strongly transmitting the new valuable qualities are all that have an especial value. Since, as a rule, thousands of their fellows must be eliminated in order to get one good plant, methods must be devised by which this work may be cheaply and effectively done on a large scale. To have many in the upper curve the line must represent very large numbers.

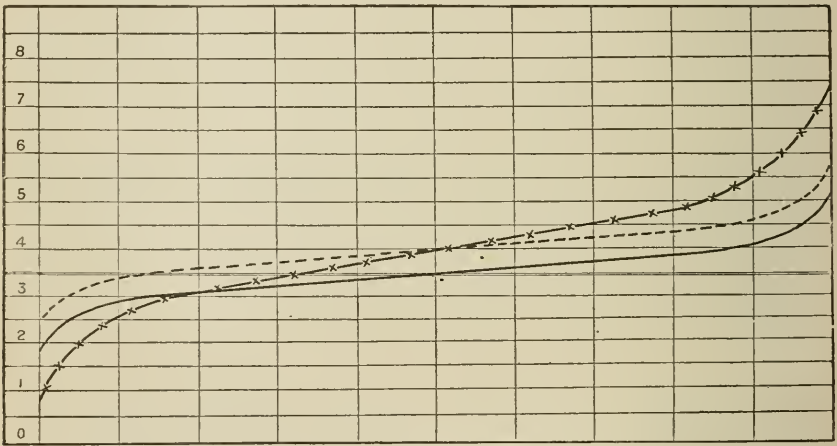

FIG. 10.—Yields of Blue Stem. Fife, and hybrid plants after further selection—all grown from seed from the best plants as, shown in fig. 9 at *o—o*. Yield of Blue Stem is indicated by the broken line, that of Fife by the solid line, and that of the hybrid by the line marked —*x*—. Each of the ten perpendicular lines stands for the average of 100 plants grown in nursery centgener.

The results of a method used for this selection in breeding wheat is shown in figure 10. The 10 plants standing highest in the Fife, Blue Stem, and hybrid curves (Fig. 9, *o—o*), are chosen as 30 mothers of nursery plots of 100 plants each, called, for convenience, centgeners. The average yield per plant in each of the 30 centgeners is a measure of the ability of the respective mother plants to produce progeny which yields heavily. These average measures can in turn be shown graphically, as in figure 10. Here it will be observed that the same general curve prevails; also that some of the progeny of mother plants whose individual yields ranked in the upper curve in figure 9 now falls below the double horizontal line. In other words, some plants which yield well individually produce progeny decidedly low in yielding power, thus illustrating the fact that all but the few in which the new quality persists must be discarded. In trying to raise the yield above the average we must eliminate not only the poor but the average blood, retaining only the blood of the few which have the greatest ability to produce progeny with exceptionally large yield.

But the important result is that some of these new stocks of seeds did give large average plant yields, showing their promise of large yields when grown under field conditions.

Even when those are chosen which yield well in centgeners there is yet need of further elimination by testing them for a number of years in the uniform test field plots. In figure 11 are shown the field yields of the three Fife, three Blue Stem, and the four hybrid stocks which show the highest centgener yields in figure 10. Even here some of those which yielded well in the nursery centgeners yielded less when grown crowded together in the grain field than the varieties used as foundation stocks as shown by their dropping below the standard line. The records of wheat breeding in the Minnesota experiment station show that the yields of new strains and varieties do not necessarily correspond with the yields of their respective mother plants.

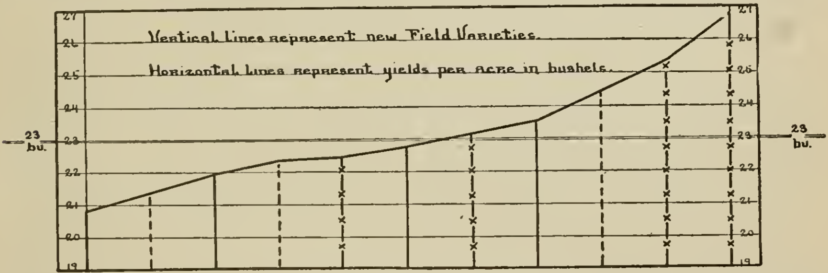

Fig. 11.—Yields, in bushels, of ten new strains of wheat developed in experiments, which have been illustrated in figs. 2-10. The curved line intersects ten vertical lines, representing the yields in averaged field trials of the nursery stocks, which show the highest centgener averages in fig. 10. The three solid vertical lines represent 3 Fife stocks, the three broken lines represent 3 Blue Stem stocks, and the four lines marked with *x* represent 4 hybrid (Blue Stem-Fife) stocks. The line representing the best hybrid strain rises almost to the horizontal line, representing a yield of 27 bushels per acre, while several strains fall considerably below the yields of the parent varieties.

In a general way two facts are illustrated here: (1) That we can improve wheat by selecting the best from our standard wheats; (2) that still more can be accomplished if we create new qualities by hybridizing and then seek from among very many those few plants which will best perpetuate the desired quality.

The real value of variation lies in the ability of the plant to produce plants which individually and in the aggregate yield more and better grain than the average of the same variety. The yield of the mother plant is a very uncertain indication of its use for the mother of a new strain, as is shown in figures 9, 10, and 11, just as Messenger's record as a trotting horse is no index to his great value as the progenitor of the American breed of trotting horses. This fact is shown with force by the history of the progeny of the Jersey bull, Stoke Pogis. He became the progenitor of a great race of heavy butter-producing cows, a purely feminine characteristic, which he could only transmit from his female parents to his female progeny.

RECORDS AND SCORE CARDS.

The careful plant breeder must have systematic methods of testing and recording the qualities of individual plants, so as to secure superior mother plants. Of even greater importance is a system for testing and recording the power of mother plants, i. e., their ability to produce progeny with high average yield, and other desirable characteristics. A good system of records should be adapted to the careful selection of the best introduced varieties; of superior individual plants within the variety; of parents producing superior young; and of resulting strains which, in numerous field trials under the prevailing conditions, prove to be the most profitable to the farmer or other grower and the most useful to the ultimate consumer.

The breeder must have a clear idea of the qualities he wishes to secure. The plant must be studied throughout all its stages of growth, cultivation, and preservation, and new as well as common uses must be understood. The weak as well as the strong points of available parent varieties must be known. Once the ideal is formed, it should be firmly adhered to, only lowered or raised where new knowledge emphatically demands or when previous improvements permit. That the breeder may secure that combination which represents the large general and ultimate value, he must clearly perceive the relative value of the various good characters.

The score card has its dangers, but is, on the whole, a most useful device for keeping records. When applied to perpetuate mere fancy points, as in modern judging of egg and meat-producing breeds of poultry, by applying to them only measures dealing with the qualities of the plumage, wattles, leg scales, and other fancy points, it is worse than useless—an obstacle in the path of progress. Its misuse in animal breeding has usually arisen from the faulty make-up of the score card, especially for those classes of animals produced for their flesh. Some valuable qualities have been given too small a weight in the scale of points, as size in the American trotter, or left out altogether, while other nonessential characteristics have often had entirely too much prominence given them. Not only has the construction of the score card been dangerously at fault, but the breeder or judge using it has often failed from not having a just conception of the relative importance of the several points enumerated in the score card. Records of performance and of qualities which can be tabulated and averaged are of the greatest value in the score card or in pedigree records of plant or animal varieties or breeds. They are measures of breeding power made up of the average records of the individual merit of the progeny.

But these weightier factors must not be too closely adhered to.

General characters or special features often appear and are seen only by general inspection. These living forms are too plastic to be brought entirely under formal yardstick or cast-iron rules or methods of selection. The breeder must adhere to a general, clearly defined plan, but at the same time he must ever be on the alert to take advantage of fortuitous variations, both among his experiment breeding stocks and among the plants grown for general crops.

In the case of such plants as wheat, timothy, clover, and in fact most of the field crops, which in the aggregate represent such a large amount of wealth, the plant is rarely seen as an individual standing out by itself where it can be compared with and chosen from among its fellows. To deal with these crops they should be planted systematically in plots, giving to each plant the same conditions for growth as to each other plant, that all may be compared and the best chosen. Here a scale of points such as is afforded on a score card provides a good scheme for comparing the various characteristics of each plant, and for making a general summarized comparison of all the qualities averaged or grouped together.

Those facts which it may be desirable later on to collect into summarized averages, or to express graphically for comparison, should, where practicable, be recorded in numbers, the unit of which should be kept uniform throughout the series. Thus, in breeding wheat it was found that the grades expressed by the ordinary terms of "No. 1 Hard," "No. 1 Northern," "No. 2 Northern," "No. 3 Northern," and "Rejected," formed a confusing mass of data after a few years, whether collected in connection with the various field tests or with the notes of individual plants in the field-crop breeding nursery. The grades are now recorded in the terms of percentage. After a series of years the grades of the respective mother plants of a given stock can be averaged; also the aggregate grades of the centgener broods of their progeny, thus giving for a series of years the average grades of one stock as compared with other stocks.

PERCENTAGE SCORE CARDS.

By giving a different numerical weight to the several valuable characteristics represented in the score card, those to which the breeder's attention should be mainly directed are set out in bold relief.

In the following score card, used in comparing strains and varieties of wheat in field tests, the attempt was made to place the larger values on those points where lies the greatest source of profit to the grower:

Basis for percentage score card for comparing varieties of wheat (*see fig. 12*).

Yield per acre	45
Grade of grain	20
Rust resistance	10
Quality of gluten	10
Amount of gluten	5
Coefficient of rise of gluten	10
	100

It will be observed that no botanical characteristics nor other distinguishing marks, nor fancy points are given a place. The whole of

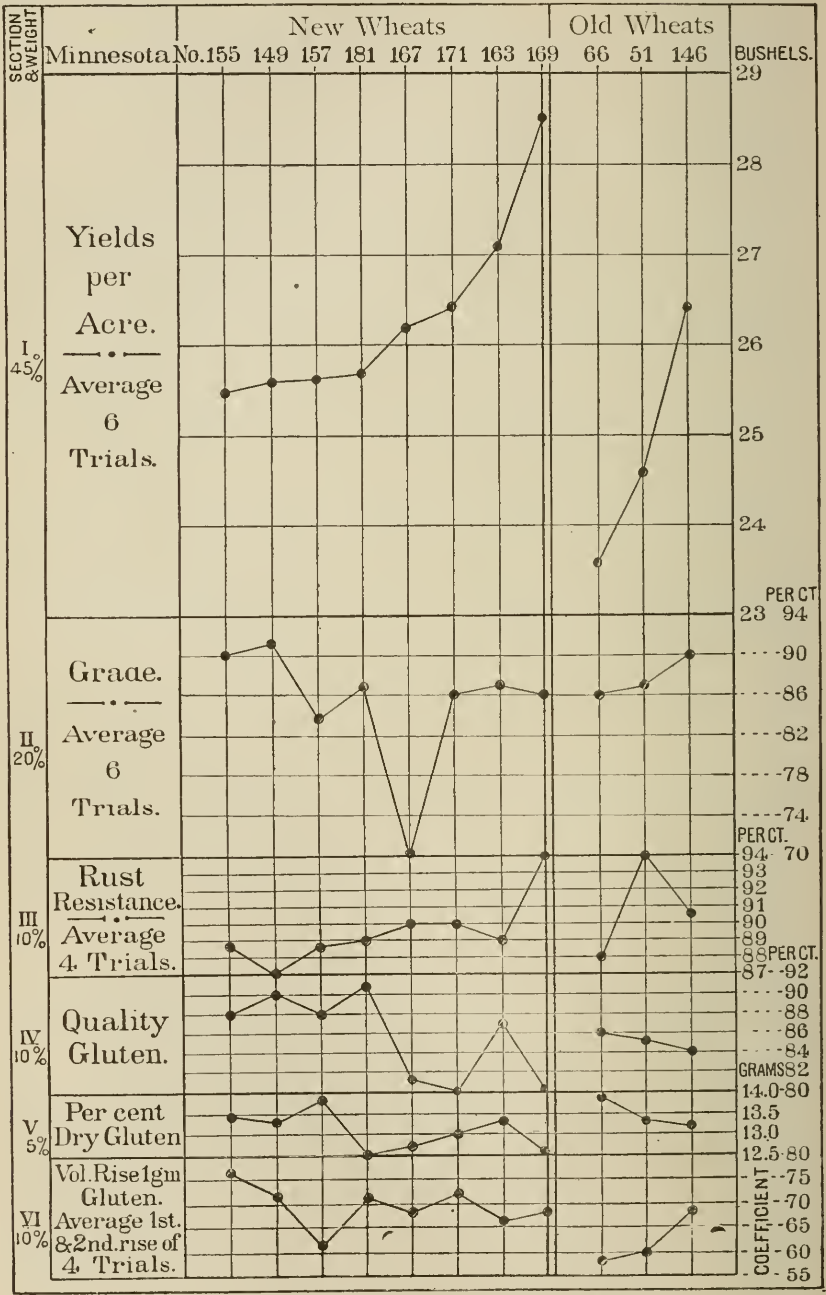

FIG. 12.—Graphic score card comparing wheats.

the 100 points of weight are given to the wealth-producing values. And the important characteristics, at least those to which our farmers at present need to give most attention, are given the largest number of points. The great need is of varieties which will yield heavier crops per acre. With the foundation varieties employed in breeding wheat, quality of the grain is much more easily secured in the middle Northwest than are profitable yields. The product per acre is not half that obtained on the old lands of England, Germany, and France. Therefore, for the present, at least, larger weight should be given in the card to yield than to other qualities.

The factors which should figure in the construction of a score card for any particular line of comparison are usually numerous, much more than is ordinarily appreciated by the framer of score cards for comparing breeding animals. And a percentage score card but crudely expresses the main truths sought.

GRAPHIC SCORE CARDS.

Graphic schemes for displaying important characteristics may sometimes be used to bring before the mind in a compact and simplified form the numerous factors entering into a comparison for the choice of a variety, a strain, or a plant for a particular purpose, or for a combination of uses. This is especially true in the case of choosing two varieties to be mated in the production of new strains or varieties. Here the difficulty is more than doubled, and some scheme is needed which will place the principal elements in juxtaposition where the eye and mind can easily see their relations. Graphic score cards could be made up in many ways and for many purposes. The fact that the size limits the number of points which can be displayed is on the whole an advantage, since the essential characteristics will thus be made to stand out more prominently, there being no room for the unimportant. Whatever form it may take, the scientific study of the score cards and their use in breeding will be found useful and interesting. Such study will aid the animal breeder to have a better conception of an ideal, and a better eye for discerning the specific points and the general value of the animal.

The percentage score card outlined above is reduced to a graphic form in figure 12 by dividing the chart into sections, giving to the respective characteristics spaces proportionate to those listed in the percentage score card. Then in these portions or divisions the several characteristics of all the varieties of wheat compared are graphically displayed. This "graphic" language, if once mastered, conveys the ideas in a much more clear and comprehensive manner than would mere words and figures.

Figure 12 shows graphically several of the leading characteristics of eight newly originated wheats, and, on the right side, three old wheats. Of these latter, Minnesota No. 51, Hayne's Blue Stem, is the parent of the best new wheat shown—Minnesota No. 169; and Minnesota No. 66, Power's Fife, is the parent of Minnesota No. 149.

The horizontal lines represent yields, grades, etc., of the respective varieties, which are given by number or name at the tops of the lines. In each of the six sections there are vertical lines representing units of the various qualities. These run only through the range of figures as given at the right ends of the lines, which include only the variations in yield, in grade, or in other qualities in their respective sections, and not the entire yield, etc.

In section 1, at the right, the yields per acre are expressed in bushels of 60 pounds each, and since the yield is the quality to which the most value is attached, the new and also the old varieties are arranged throughout the chart in the order of their yields.

In section 2 the grades are expressed in percentages, thus making the comparison of yield and grade comparatively easy in the two sections.

In section 3 is given the relative rust resistance of the several varieties, as expressed in percentages of rust showing on leaves and stems during ripening.

In section 4 is given the quality of gluten as determined by an expert in stretching and manipulating the gluten from which the starch had been washed out with cold water. (See Pl. I, fig. 1.)

In section 5 is given the percentage of dry gluten in the flour.

In section 6 is given the quality of the flour as determined by the bakers' sponge test (Pl. I, fig. 2), expressed in the volume of loaf produced by each percentage unit of gluten. This is obtained by dividing the grams of dry gluten in 100 grams of flour into the volume of loaf of dough produced from the hundred grams of flour. These figures represent the averages between the volumes of the first and second rise of each kind of flour.

This chart is an aid in the selection of those varieties between which hybrids are to be made. The new wheat known as Minnesota No. 169 stands out prominently as the best wheat, and as one of the parents to use for crossing to produce further desirable variations which may serve as foundations of new strains or varieties. Minnesota No. 163 is the next best yielder, has grain of good grade, is fair in rust resistance (a point in which No. 169 is very strong), is good in quality of gluten, very good in the amount of gluten, fair in amount of loaf a given amount of gluten will make, and should serve well to cross with the large-yielding No. 169.

Minnesota No. 171 would also be a promising wheat for hybridizing with No. 169. No. 167, on the other hand, while nearly as large a yielder as No. 171, is deficient in grade of grain and in the quality of its gluten. Minnesota No. 149, though not so large in yield as some others, grades high on the market, has a relatively large amount of gluten of very superior quality, and would rival No. 163 to hybridize with No. 169.

METHODS OF PLANT BREEDING.

RELATION OF PLANT INTRODUCTION TO PLANT BREEDING.

In addition to the limited number of native plants suitable to special cultivation in each locality, every country finds it advantageous to introduce plants from other countries, and to transfer plants

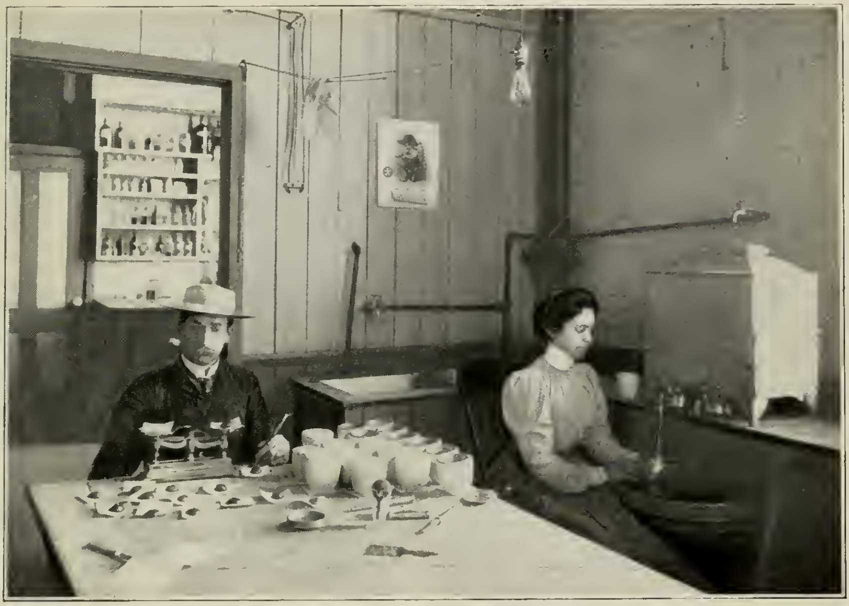

FIG. 1.—TESTING THE GLUTEN OF NEW WHEATS AS TO ITS AMOUNT AND STRENGTH.

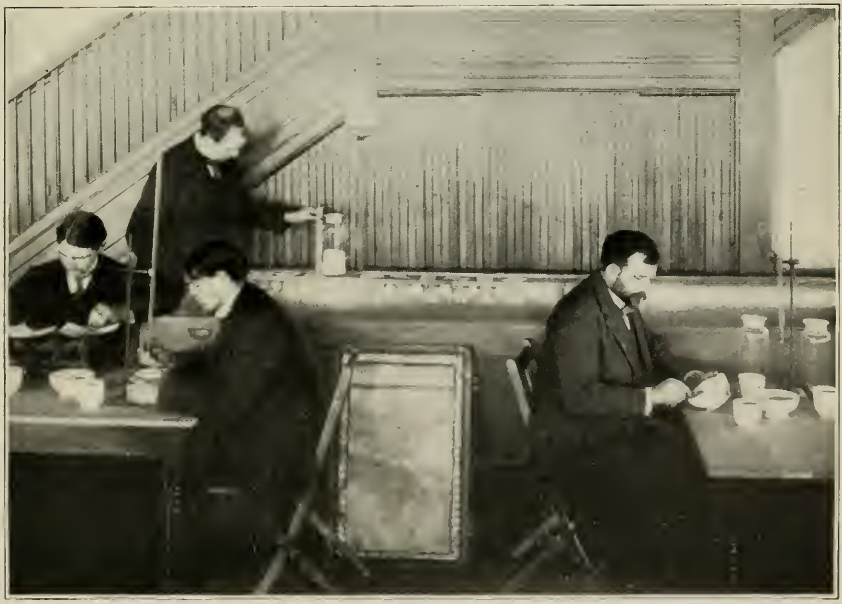

FIG. 2.—MAKING THE BAKERS' SPONGE TEST OF NEW VARIETIES OF WHEAT.

from one district or neighborhood to another. The number of foreign plants which the Department of Agriculture is introducing into the United States through its trained agents is very surprising to one not acquainted with the facts, and there is a constant transference of varieties within the country to localities new to them. These agents, by becoming acquainted with the various local conditions of our own country, and then seeking plants in foreign countries having similar climate and soil, are able to understand the needs of each locality and to introduce plants which will thrive and become useful. As illustrating the extent of the work in this line which it is practicable to do, it may be mentioned that in Minnesota several hundred kinds of wheat have been introduced, mainly by the experiment station, and somewhat lesser numbers of several other cereals; also numerous species and varieties of forage and root crops, besides hundreds of varieties of apples and other trees, small fruits, and flowers. From the station the most promising varieties are transferred to the substations, to a dozen trial stations distributed throughout the State, and to many farms from which reports are required. And when it is observed that a somewhat similar work is maintained in each of the States and Territories of the United States, it may be seen that plant introduction is a prominent feature. With the national Department of Agriculture encouraging, leading, and directing the main features of this work, the whole is held together in a helpful way, and good results are being realized. Seed and nursery firms, private individuals, and agricultural and horticultural societies are also doing much to assist in adapting varieties to special conditions.

That this line of helpful work is not overdone is shown by some of the recent beneficial results. *Bromus inermis*, an important forage grass for the drier areas of the Northwest, and Dwarf Essex rape, a forage plant suited to providing succulent forage in early and late autumn, are examples of new crops of very great value in the Northwest. The tea and the many new plants from the Mediterranean regions which are being introduced into the South and Southwest by the Department of Agriculture illustrate the fact that there are regions elsewhere from which only part of the plants have been heretofore brought to this country for thorough and fair trial. Each of the varieties now existing in each locality in other countries and those being bred for special conditions by our foreign friends may fit into some locality, broad or limited in area as the case may be, in our own country. And, likewise, plants which are so changed as to suit special environments in our country may find their special niches in one or more foreign countries. We have a case in point in Indian corn. In America this crop has been bred for numerous conditions. Early, large-yielding dent varieties have finally been bred for southern Minnesota and other Northern States. In Europe only those large-yielding varieties of corn from the South have been tried, and they have been pronounced a failure. These cold-region varieties have not been tried, but would probably succeed well for growing fodder and silage; but of even greater importance is the fact that these varieties would be the best foundation stocks to utilize in breeding kinds of corn suited to growing the grain as well as the fodder in many European localities.

Liberality of plan in introducing and testing new plants is the part of wisdom. In not a few instances plants have not at once fitted into their proper niche, and some useful kinds have been wrongfully discarded. For example, *Bromus inermis*, mentioned above, was tried by several experiment stations and by numerous farmers throughout the middle West for a number of years, and no one saw in it anything of especial promise. Attention was brought to it by the Canadian experiment stations at Brandon, Manitoba, and at Indian Head, Assiniboia, and, upon further and more extensive trial, it soon became popular in the Dakotas and adjacent States. Its recent wide distribution by the national Department of Agriculture, aided by experiment stations of the interested States, is proving to be of great value.

Some introduced varieties and species which seem, as compared with the better standard sorts in any district, to be of too little value to be useful, may be so changed as to fit their new conditions, or they may have special uses not commonly regarded. Thus, some of the hardy Russian apples introduced throughout the middle Northwest by Prof. J. L. Budd and others may prove useful only as a source of hardy apple "blood" in the formation of hybrid races which will endure the severe winters of this northern climate. Mr. Luther Burbank, of California, has introduced into his plum-breeding nursery many types of plums, which he crosses or permits to cross in all conceivable combinations, that he may secure seedlings of types almost innumerable, from which he may select the few which are really valuable. Some forms of Speltz wheat have not proven to be a profitable crop in Minnesota, yet a small infusion of their blood into the so-called Blue Stem variety may be of value. The Blue Stem shells out badly as soon as ripe, while the Speltz has chaff which remains firmly attached to the kernel, and a little of the Speltz blood might be used to correct the greatest weakness of the otherwise very good Blue Stem variety.

CHOICE OF FOUNDATION STOCKS.

The plant breeder wins half of the battle when he secures suitable foundation stocks. To do this often requires the extensive testing of introduced and standard varieties in the locality and under the conditions for which the effort is to be made to breed improved varieties. But as a rule there are standard varieties commonly and successfully grown in the vicinity which will serve until, by further variety testing, better stocks are secured. The earliest efforts at breeding any species are often experimental as to the methods to follow, and a mediocre foundation stock will serve at least to practice upon, so that when the better varieties have been found the work may be undertaken in a thorough-going manner. There are many matters of detail to learn, and the most practical way to master these details is to begin the actual work at first on a more modest scale than is advised for the general work later on. As the work progresses the testing of introduced and standard varieties and the results obtained from new kinds—if full records are kept throughout—will show from which foundation stocks it is the best to try to make new strains or varieties. It occasionally happens in the experience of the observing breeder that some chance form will be found which is so much nearer the ideal for which he has been systematically seeking that he should use it for his foundation stock, discarding all or most of the products of his former labors. It often pays to seek long and far for these unusual forms and individuals among the plants of a wild or native species that the best available foundation stock may be secured.

NAMES AND NUMBERING OF VARIETIES.

The nomenclature of varieties of cultivated plants presents many vexing problems. Seedsmen are prone to multiply names by creating synonyms for new or even old standard sorts, that they may have what at least appear to be new varieties for their customers. On the other hand, the class name too often adheres to the selected stocks in cases where improvement in yield or other quality has been produced, but no visible botanical change has been made; and the new strain, having no special distinguishing mark, is classed with, and probably wrongly condemned with, the parent variety. And in many cases in wind-pollenized species, such as corn, a once distinct variety is changed by admixture with other varieties, and what is known by a certain variety name in one locality is soon quite different in value or in appearance from what is known in another locality by the same name and which had, in part, the same origin.

Variety testing, an essential part of the experiment station work, is an invaluable adjunct to the seed and nursery business, and is advantageous on the farm and in the orchard and garden. It is necessary to test varieties that we may know which produce best, and also that we may be able wisely to select foundation stocks for the work of making better varieties by breeding. Unless the figures or general statement of results regarding the test applies to a definite variety or stock of seeds it is valueless, or even may be misleading and harmful. The principle of cooperative testing of varieties by experiment stations is properly growing with rapidity, since experiment stations which are equipped for this work can not only do much of it better than growers, but they can greatly save in the aggregate expense. There is a part of this work, however, which must be done by the grower, since all his conditions can not be duplicated on the experiment farms.

The Minnesota experiment station has adopted a system of numbers for varieties, using variety names more as class names. This plan grew out of necessity. Several superior strains or subvarieties of Blue Stem wheat and Fife wheat, for example, had sprung up among the farmers and at the experiment station. These could not be clearly identified by any botanical characteristic, but some of them were superior in their yield.

The following general plan has been in operation for several years and has given excellent satisfaction:

(1) Whenever a sample of seed is received from any source it is given a number with the words "Minnesota No." prefixed, written thus: "Minnesota No. 169 Wheat:" "Minn. No. 13 Corn," etc.

(2) In a book called the "Minnesota number book" each variety is entered separately, and its subvarieties are numbered consecutively as they are received by the station and its substations.

(3) Whenever a new subvariety is taken from the field-crop nursery to the uniform-test field plots, it also is given a number, taking the next number to the one last entered.

(4) In the Minnesota number book are entered in appropriate blank forms all facts obtainable regarding the name, source, and origin of each particular sample of seed which may form the basis of a new variety.

(5) A small number of the plants of a newly introduced strain or variety are grown, and full descriptive botanical notes are recorded under each, that its identity may not be lost.

(6) For our purposes the number, with the words "Minnesota No." prefixed, thus becomes the name of the strain or variety, and in case it is of our own breeding there is no other name given, and the experiment station desires that no one else give it another name.[1]

(7) By keeping this number and the identical stock of each seed together inviolate we can attach to it a performance record. And the association of the name, the record for large yield, and the identical stock or variety which made the record, is what gives peculiar pedigree value to the seeds.

(8) This plan of numbering, or naming with numbers, is useful in cases where a variety is improved from year to year, since when the old no longer represents the improved variety it can be given a new number. Thus, Minnesota No. 13 corn has been sold to the farmers for several years. During each year a field of stock seed has been grown, called our "seed-corn patch," in which this corn has been rigidly selected for better yield, but not to change the appearance. The seedsmen and many farmers are now annually selling large quantities of Minnesota No. 13 seed corn, some of which is from seed sold by the experiment station in the years when the improvement of this corn had been only begun. We shall now give our improved stock of this variety a new number (Minnesota No. 172), and advertise it as an improved progeny of Minnesota No. 13. Names being easier to remember

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[1] Seedsmen should not give a name of their own choice to a variety with such a record of performance, and thus lose the connection between the name, the performance record, and the seed, or doubly violate good ethics by appropriating to themselves both the name and the experimental record, for which only the experiment station which tested it can stand responsible.

than numbers, they are better in such cases, as fruits or potatoes which are reproduced by cuttings or grafts, where the variety can be definitely described botanically. It is expected that occasionally, when a newly originated variety of field crop especially distinguishes itself for peculiar value, it may be given an additional name as an especial distinction of merit.

(9) Since many very valuable new varieties never gain commercial recognition, and their originators secure neither credit nor profit, the need of adequate business methods in installing among growers each valuable new variety is of great importance. Experiment stations and the General Government need plans adapted to each new class of varieties originated, as do also seed firms and amateur breeders. Gift packages accomplish this in some cases. In others useful new varieties are thus discredited and wholly or partially lost. Strong seed merchandising firms and large numbers of experienced seed growers are most important agencies. The Minnesota experiment station has had most encouraging success with its plan of distributing no gift packages, but selling under pedigreed certificates to picked growers of seeds, each of whom becomes a propagator for profit. The station thus fosters the business interests of its many cooperators. Their desire to retain the opportunity to cooperate in introducing successive new varieties stimulates these cooperators to employ good methods and fair dealing.

BREEDING BY SELECTION.

The general plan to pursue in breeding plants is here divided into two sections, in order that selection may be dealt with before taking up the more complex question of combined hybridizing and selecting. Selection is of two kinds: (1) Selection of seedling plants, and (2) bud selection.

SELECTION OF SEEDLING PLANTS.

Most of the variation in plants has its origin in reproduction from seed, the variation being, as a rule, the greater the more distant the relationship between the two parents. This variation shows strongest among the seedling plants, and to a much smaller extent among the plants produced from the buds or cuttings taken from the seedling plant.

While half the battle is won by choosing the variety to serve as a foundation stock, more than half of the remainder is often won by the first one or two years' work in selecting mother plants within the chosen variety. For example, the writer, in starting to breed from several varieties of flax, used 100 seeds from the bulk grain of each of seven varieties. The plants were grown singly in hills a foot apart each way. It was desired to choose from among the plants of each variety one plant for a mother of a stock to be developed into a strain or variety yielding a large amount of seed per acre, and a second plant to serve as the mother of a tall-growing strain or variety for fiber. Upon inspecting the several plots the range of choice among the individual plants was not nearly so great as had been anticipated. There were no good types of heavy-yielding plants, and few, or none, remarkable for their height.

In the same field with the crop nursery the seven varieties of flax were growing in large field plots. There was occasionally a very tall plant, and by diligent search plants were found which had a very much branched top, and were apparently heavy seed bearers. A tall plant and a heavy seed-bearing plant were chosen from each variety. The next year 100 seeds were planted from each of these 14 mother plants. The plants from the tall mothers averaged several inches taller than those from the heavy seed-bearing mothers, though the latter had not been chosen for low stature, and the supposition is that they were of average stature among the plants of the respective varieties from which they were selected. Thus, the first year the selections were made among very large numbers, and the second year the mother plants were tested by comparing a large brood of the progeny of each.

In the accompanying illustration (Pl. II, fig. 1) are shown the results of the experiments in the improvement of flax by selection:

The bundles at the right in the lower row are several samples of flax bred for branching tops with many seed bolls, no effort having been made to change the height by selection.

The six bundles at the left have the same parentage as the seven in the same row, but were bred by selection for greater length of straw. All the bundles of this row are from plots planted at the rate of over 2 bushels of seed per acre, as in growing crops of fine fiber.

Seeds from the same stocks were planted thinly, less than 3 pecks of seed per acre, as for growing crops of flaxseed, and bundles of the resulting plants are shown in the upper row.

Whether grown thickly or thinly, the newly bred qualities of tall growth and fine stems showed in the plants grown for fiber, which stood several inches taller than those grown for seed, which remained the same height as the plants of the original parent varieties. The proof is very strong that long, fine fiber can be grown in the dry climate of the Northwest. Flax bred to grow tall will make fiber in Minnesota closely approaching in length, fineness, and strength that grown in western Europe under moister conditions.

The most important feature of this experiment to Minnesota is the yield of the flax bred to grow seed. The tests of yield for seed have not yet been completed, but since such a profound change has been made in the height of those grown for fiber it would be very strange did not some of the strains shown on the right prove very heavy yielders of seed.

From some of the best of these mother plants the experiment station now has seed, as yet only in sufficient quantity for field tests, of seven strains of tall-growing kinds of flax, and seven others which are apparently superior in their yield of seed. If the stocks first planted in the field-crop nursery (among which relatively little variation was found because of the small number) had not been discarded and had the mother plants been chosen from among them instead of from the fields, where there was a large number to choose from, it is probable

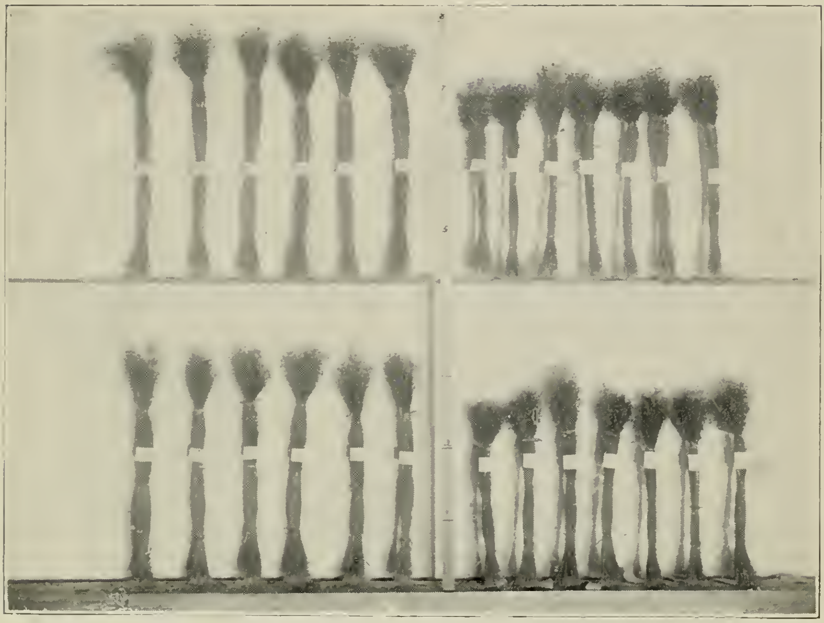

FIG. 1.—IMPROVEMENT OF FLAX BY SELECTION.

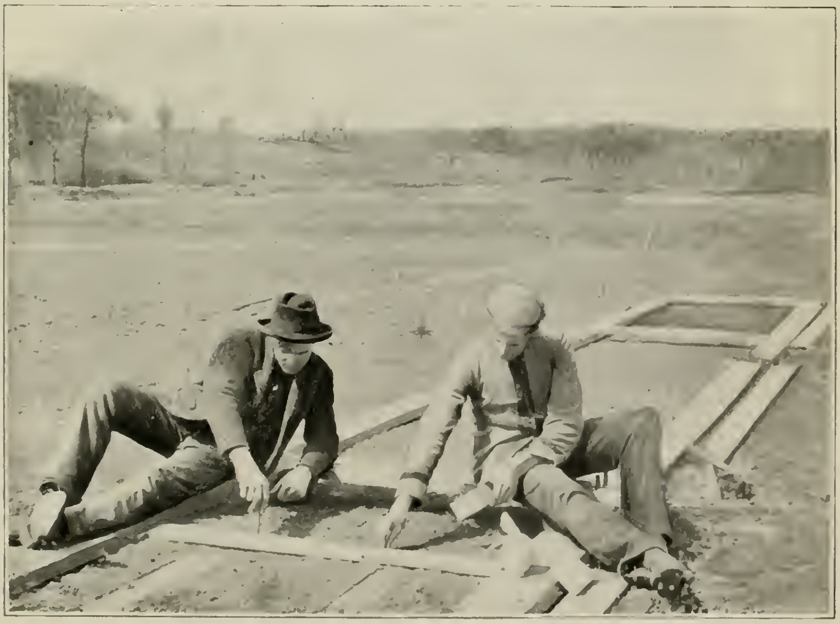

FIG. 2.—PLANTING SEEDS IN THE CROP NURSERY.

that progress in making useful new varieties would have been very much slower. It was practicable to select choice plants from the field of flax, because each seedling plant stands out distinct on its single main root, while it is not practical to thus select in case of wheat, which sends up numerous culms from each seed, the culms from two or more seeds often being interwoven.

While the general principles of breeding are the same throughout, their application must vary greatly, according to the habit of the species dealt with. One plan is best with open-fertilized species, another with close-fertilized species. Species freely cross-pollinated by the wind may require different treatment from those occasionally crossed by insects. Varieties propagated mainly or wholly by cuttings require different plans from varieties propagated by seeds. Annuals often require a different method of selection from that used with perennials. In case of varieties used in one locality which are produced from seeds or bulbs grown in another place, the breeding requirements differ from those where the seeds are bred in the same environment under which the crops are to be grown. The whole range of special means and requirements for breeding the thousands of varieties for many localities having different conditions can hardly be more than touched upon. Further on a somewhat detailed plan for breeding several crops will be given as examples, showing how the systematic improvement of a plant may be worked out.

BUD SELECTION.

While efforts should be mainly directed to a choice among seedling individuals, yet the variation among the buds, branches, spikes, or heads of the plants is sufficient to warrant general and in some cases special attention. While we are warranted in planting the seeds of wheat singly in the hill, that we may judge or test the weight and quality of the crop of seed from each individual plant so as to secure the best plant, we should also secure the seeds from the best spikes. And in case of many flowers multiplied by cuttings, where bud variation is sometimes considerable, marked improvements may be made by bud selection. Each branch, leaf, and flower springs from a single bud, and, though bud variations do not generally breed true to type, they so often do that the selection of marked bud variations is well worth attention. Sometimes a limb, a flower, or other new bud feature does not breed true at once when reproduced. By growing many plants from the peculiar branch the same bud variation may appear on one or more plants, and upon following up nature's hint workable ore may be discovered deep down in the mine. Sometimes we find seminal variations, and more frequently bud variations, which breed true, but as a rule we secure them wavering, as if staggering under a load of inherited tendencies which are trying to reduce them to the level of their species, variety, or family. As the special school takes the youth from among the steady-going things of the country or village home, develops his latent faculties in some line of art or science for which he has shown a bent, and fixes the habits and ambitions of his life on some specialty, so we must discern new powers in plant and animal life, and by means of environment (or by cross-breeding or hybridizing) give it development, and by extensive and intensive selection fix it as a type. And we can not stop with having built up new types. We must not even be content with letting them stand still. The saying that "man can not stand still—he must progress or he will go backward," is as true of improved varieties of plants as of men. Give the Indian boy an education, even educate him as a specialist, and place him back among his own uncivilized people, and he will generally return to nearly their standard of living. The higher a variety is specialized under the art of the breeder and under the nurture of improved conditions, the more difficult it is to keep up its high qualities. Not only must the high nurture be kept up, but the selection also must be continued, or retrogression toward the old primitive type may be expected. Breeding upward specializes a variety of plants or animals for definite conditions, and to secure the full value of the improved blood of a plant or animal it must continue to be kept under those good conditions.

From formal experimentation we may not produce the greatest wonders. These may arise as accidental variations in the course of common plant production. In fact, most of the varieties we now have were originated by picking up these odd forms, and from a small beginning, persistently followed up, greater variation was produced and then fixed to uniform types. The numbers of plants and animals bred under the varying conditions of common production are so vastly greater than the number which the breeders of plants and animals can deal with that it is not strange that a larger number of useful variations arise there than in the small fields and farms of the professional breeders.

As knowledge among men accumulates from generation to generation, there is more impulse for still greater development of thought. The thought of one decade is not only a basis for better thought in the next generation, but it is an exciting cause of new and more complex thought. The throwing together of new thoughts somewhat related, yet distinct, excites the creation of more thoughts. So, while improved varieties are a higher basis upon which to build other new varieties, each pair of varieties which are specialized so as to be very different in character, though related, have within them when crossed the inciting causes, the power of creating entirely new variations. The principles and physical relations or changes of the brain cells involved in the physiological phenomena mentioned are the subject of research as well as the principles and the physical changes of the generative cells in the generative phenomena. The obscurity, if not other features, common to the phenomena of mind and of heredity have led some plant breeders to believe that the mind of man has a direct relation to and influence over his work as a plant breeder, and that some men have a greater power in changing plants than others. When due credit is given to the great care, to the use of immense numbers, to the acute observation in detecting new things, and to the system used in developing, testing, and comparing many new types by those who are most successful in producing wonderful and useful forms, there is so little room for the special direct influence of the mind of the plant breeder that it needs little consideration.

BREEDING BY HYBRIDIZATION AND SELECTION.

In breeding by selection alone the variations occurring naturally or accidentally within the variety are depended upon. Man simply selects the choicest. These are usually placed under such environment as will cause desired characteristics to develop best and to stand out. In breed or variety formation through the agency of hybridization, followed by selection, man plays almost a creative part. Where there is no variation of such nature as desired, it is created by bringing together two of the many forms which have varied from some ancestral forms, yet not so far but that they will cross-fecundate. The further they have departed from ancestral characteristics and formed diverse qualities, the more likely will their progeny exhibit new characteristics made up by combining those which have become so radically different in the two parents. In a group of men conversing about a subject concerning which they have all practically the same knowledge few new ideas are given to each mind in the group, and few new thoughts are created through suggestion to each mind. They separate each with the same knowledge and beliefs as before. But if these men begin conversation about a subject concerning which they have different ideas and beliefs, each mind not only receives new ideas, but the creative power of the mind develops new thoughts, theories, or interrogatory notions, which may or may not be rational. So, when two nearly related plants or animals are crossed, variation is not so much excited, and the progeny are very similar to the parents. But when two plants widely different are hybridized, the natural tendency to vary develops new combinations or apparently creates characteristics, new in kind and degree, which hardly seem to be the combined results of any two characteristics in the parents. Since this comparison seems so useful in carrying to the mind a conception of the nature of heredity and variation, it may properly be carried a step further. The human mind has been built up step by step from the minds of semicivilized races of the past by the association of ideas, resulting in the creation of the more complex knowledge of the present. So the varieties of plants and animals are being built up by the creative power of natural variation. And, as printed pages, the school, the modern railway and steamship, and electrical agencies are furnishing an environment in which the mind develops and new ideas are created more rapidly than ever, so the plowed field, the fertilized soil, the isolated position of the cultivated plant, giving it more nourishment, and especially the bringing together and crossing and hybridizing of various forms of plant life, by accident and by the intentional effort of man, furnish conditions under which varieties are improved and new characteristics are created.

While the American genius for inventing machinery was being developed, indian corn and the American trotting horse were being most wonderfully evolved into higher types. While the Japanese mind has been rising out of the lethargy of ages and becoming a valuable factor in civilization, the sugar-beet plant has been changed from a species which had held its characteristics through an epoch of history to a type profoundly different and of enormous value. As the thought of modern Europe has been centered in America through the migration thither of many of her most progressive people, so Burbank in his garden, and in a broader way all of our farmers and experimenters, are centering the blood of all the valuable plants of the world into varieties which shall improve plant life in our country. When we view the development of the mind of the human race from its original state to its present achievement we can not predict a limit to its expansion in the future. Nor when the development of useful plant and animal forms, through their changes from a simple beginning to their present complexity, is observed, can we assume that there is any practical limit to the betterment of our plant varieties. So far as the present generation is concerned, there is ample room for useful improvements in all classes of economic plants and animals. Since by breeding the value of some plants, as sugar beets, has been enormously enhanced, it does not seem too much to hope that most of our economic plants can be made 25 per cent more valuable than now.

DEGREE OF RELATIONSHIP IN CROSSES.

"In-and-in breeding," "outbreeding," and other expressions relating to the close or distant relationship of parents have been prominent subjects among animal breeders. Charles Darwin's dictum that nature causes benefits to arise from crossing and abhors self-fertilization has been a foundation stone for the theories of writers and teachers upon animal breeding, though many men are skeptical about the application of this statement to some of the many conditions in animal breeding. Plant breeders have even a greater range of conditions as to the degree of relationship between parents, because they deal with the self-fertilized and partially self-fertilized species, as well as with those requiring or allowing of the union of the germs of two parent plants. Possibly Darwin's law would more broadly cover the truth if expressed thus: Nature abhors a radical change which would require species to cross in much closer or in much more radical relationship than is their long-established habit.

It is known that very close breeding among poultry, swine, human beings, and in corn is harmful. On the other hand, wheat and other self-fertilized species, though bred up to a high standard of specialization, as in the production of a large proportion of seeds as compared with the remainder of the plant, do not seriously, if at all, retrograde under self-fertilization, the most incestuous kind of inbreeding, carried on for many generations. And the evidence is most conclusive that some of these close-fertilized species may be materially improved by a system of selecting the self-fertilized plants. The best newly originated variety of wheat at the Minnesota experiment station came from a single mother plant (or may we call it mother-father plant?) chosen in 1892. There is some good evidence also that close breeding in some classes of animals is not injurious, and in many families and herds of animals close breeding, or in-and-in breeding, has been very useful in fixing valuable new types.

The general statement that cross breeding gives increased vigor, size, and value is also too sweeping, and the limitations of the good effects should be better understood. Crossing does often increase vigor, size, and other good qualities, and it often decreases these qualities, and sometimes in radical crosses the average progeny is exceedingly weak, even being so weak in fecundity as to be sterile. This fact was observed in certain wheat hybrids at the Minnesota experiment station, where in a few generations the hybrid stocks became very weak and finally ceased to produce seeds, while other stocks from the same two individual parent plants were very strong and were the progenitors of some of our most promising new wheats. But, so far as the writer has observed, hybridizing increases variation in the first few generations. Swingle and Webber[1] show that many radical hybrids vary but little the first generation, but that all hybrids vary within a few generations. Usually this variation is both upward and downward, though in some cases of radical crosses none of the progeny are equal to either of the parents, and in other cases nearly all are better. As to whether the average progeny of the cross is stronger or weaker than the mean between the parents—"mid-parent," as Galton expresses it—depends in part on whether the parents are properly related, or whether the cross is too radical or too close to suit the habits of the parents. Determining what degree of relationship is best in the mating of plants is, indeed, an interesting subject for scientific inquiry.

In hybridizing plants to form new varieties, large numbers can usually be employed, and the average qualities of the progeny is a matter of no particular consequence. The important feature is that the hybrid stocks vary greatly in the desired direction, giving a few plants which, when multiplied into varieties, will average better than the parent varieties. No matter should all but one in a thousand be discarded, if that one will produce a race of progeny with the improved qualities. With this theory of hybridizing and the practice of making a very large number of crosses, giving each a few years or generations in which to show the tendencies of its progeny when grown in large numbers, and with a systematic plan of eliminating, the use of hybridizing in variety formation becomes a practical and very powerful agency in plant improvement.

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[1] Yearbook, U. S. Dept. Agr., 1897, p. 407.

HYBRIDS AND CROSSES DEFINED.

Very much has been written concerning hybrids, and this literature contains the results of many experiments. Swingle and Webber have summarized the facts well.[1] They use the word "hybrid" to mean a plant resulting from cross fertilizing plants differing in their relationships, whether that difference is great, as in species or even in genera, or comparatively slight, as in distinct varieties. Cross-bred plants are those which have resulted from the cross fertilization of plants within the variety but separated in descent by at least some generations of seed production. Self-fertilized plants, on the other hand, are those which have resulted from the pollen of the same plant impregnating the flower, including those plants which have arisen from buds and cuttings from the same seminally produced plant.

IMPORTANCE AND METHODS OF HYBRIDIZING.

The operation of cross pollinating in hybridizing is easy in the case of most useful plants. This work forms but a small part of the work of variety formation, most of the labor and expense being connected with collecting and testing to find superior foundation stocks, with the growing and selecting of large numbers of hybrid plants and with testing the resulting varieties that only the best may be propagated and disseminated. The methods of hybridizing wheat, corn, and apples, and a few other plants will be given briefly on future pages, as illustrative of the work with some classes of plants. It is necessary fully to understand the structure and habits of the flowers of the plant to be dealt with, but no great skill or profound knowledge need be attained, and the necessary appliances are few and simple. In case of crosses between nearly related plants there is often little more variation than among self-fertilized plants. Where varieties not too distant in relationship are hybridized the variation, according to Swingle and Webber, usually shows in the first as well as in the succeeding generations, but where the relationship of the parents is very wide, as between species or genera, the hybrids are more likely to be intermediate in appearance between the two types for the first generation. By self-fertilizing or crossing the flowers on these plants of the first generation greater variation is produced, and, for a few generations, continued new forms may be expected to result from the variation inaugurated by the hybridizing of the two species or genera. Variation can often be profitably increased by crossing the hybrid back on one of the parent species or by crossing two hybrids of different or partially different parentage. In some cases hybrids persist in remaining intermediate between the two parental forms or in taking and retaining the characteristics of one or the other of the parents. In this case the only utility of the hybrid may be to serve as one parent to use in making still another hybrid; or it may be wise at once to discard it, that more attention may be given to hybrids which show variation. Garton Brothers, of England, who have done most commendable work in producing hybrids among field crops, say that "the effort should be to secure a reaction," meaning that those crosses are most desirable which cause excessive variation or a multiplicity of types, some of which will probably be found useful new forms from which superior varieties may be developed. Where the relationship is very distant the hybrids are likely to be weak in fecundity or in some other vital characteristic, though progeny of great value sometimes occurs.

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[1] Hybrids and their Utilization in Plant Breeding. Yearbook, U. S. Dept. Agr., 1897, pp. 383–420.

BREEDING FOR SPECIAL AND NEW USES.

While our best efforts should be directed toward better fitting our staple crops and our commonly used plants for their accustomed fields of usefulness, there are special and new uses for plants which should receive attention at the hands of plant breeders and plant introducers. This work, which was emphasized in previous paragraphs, can be accomplished in part by selection, but even more thoroughly in most cases by hybridization, followed by selection. To extend the growth of some species to regions in which they do not now succeed, varieties are needed which will endure more cold, or more heat, or more drought, or more wind, or more alkali, or be adapted to other features of new surroundings. Many of the changes needed for these purposes could not be bred into the varieties, especially in case of close-fertilized species, by selection alone. New varieties must be created by hybridizing. The work is sometimes quickly accomplished, and sometimes results come very slowly or not at all. Resistance to disease is a quality of superlative importance in many instances. Sugar beets have been enriched in their percentage of sugar content, and made more valuable by lessening the amount of solids other than sugar which are expensive to remove in the manufacture of pure sugar. Since sugar beets are an open-fertilized species, the natural crossing of the plants has doubtless been a powerful agent in creating this new value in the roots. Corn is being made a better food by increasing its content of nitrogen compounds, collectively called protein.

BREEDING NITROGEN INTO FIELD CROPS.

There are few general considerations in the breeding of plants and animals which are more important than that of breeding a stronger tendency toward the production of nitrogen compounds. Protein is worth, on the average, about 4 cents per pound in those substances used for food for animals and man, while carbohydrates and fats, excepting in special forms, as in highly flavored butter, are worth much less. The farmer can produce starch, cellulose, and sugar in his staple field crops for a small fraction of 1 cent per pound, often a very small fraction. The carbonaceous substances contain neither nitrogen nor mineral fertilizing substances, and their use as plant food is limited to improving the water-holding power of the soil, and, upon decaying, furnishing active compounds which help to elaborate plant food from the insoluble mineral and nitrogen compounds in the soil.

On the other hand, the protein of our food plants, in addition to being very valuable as a food, is a most important fertilizer. In addition to its value of about 4 cents per pound as a food stuff, protein compounds are worth, at the commercial rate of nitrogen, about 2 cents per pound as fertilizers. Assuming that half of the nitrogen can be retained for fertilizer on the farm upon which the crops of grain and forage are grown and fed to animals, we have 1 cent per pound valuation of the protein in the foods we raise for our animals as a manure to add to its value of 4 cents per pound as a food for live stock. These values can not all be secured and realized by the farmer, because he sells some of his crops in the cities. We should seek to increase the yield of nitrogen per acre, as well as the total yield of crop, just as the breeders of sugar beets must increase the yield of beet sugar per acre rather than the tonnage of the roots, and the breeders of dairy cows increase the yield of butter rather than the yield of milk. Thus we should add to the protein content of our cereal and forage crops. In case of corn fodder there is a special reason for increasing the content of protein. The varieties of corn used for dry fodder or for silage already yield so well that a superabundance of roughage can easily be produced. But to make it valuable as a balanced ration we must add to it expensive concentrated foods, such as bran, oil cake, or other grain products.

By increasing the percentage of protein in the fodder or silage a less amount of the expensive grain foods will be required, and, the ration being cheaper, will leave a larger margin of profit. But of even greater importance is the breeding up of the nitrogen content in clover, cowpeas, alfalfa, and other plants which gather nitrogen from the air. These crops should not only have their nitrogen content increased, but they should also be so bred as to succeed far better than now and under far wider ranges of conditions. Red clover, for instance, has been improved comparatively little since it was brought from Europe. It thrives well under some conditions, yet it does not meet all the difficulties and is not profitable in some localities where it should be made very useful. If it could be grown under conditions where it is not now hardy, its use as a fertilizing agent would be greatly extended, and if it were bred to extract still larger amounts of nitrogen from the air, it would be more valuable as a fertilizer and also as a food for domestic animals. Mr. W. T. Swingle proposes that the nitrogen-gathering bacteria associated with the nodules on the clover roots could also be bred so as to be more actively useful; and since brewers have successfully bred special varieties of brewing yeast for making beers of different qualities, the breeding of these bacteria would seem also to be a practical undertaking. The field pea, likewise, is a crop worthy of most serious effort, both that varieties may be secured which will produce profitable crops in localities where it is not now successfully grown, and that the contents of its seeds and its vines and leaves may have a larger percentage of this most valuable element, protein. Alfalfa, cowpeas, and soy beans, for like reasons, should be improved. These are the five principal nitrogen-producing plants of this country, each with its special very large field of usefulness. To change each plant so that its range of successful production would be enlarged 10 per cent, its protein content increased 10 per cent, and its yield increased 10 per cent where now grown, would cost only a very small fraction of the resulting increase in value. Increasing the protein content in this manner would in the aggregate be a very large increase of the nitrogen annually gathered from the air into the soil of the country. Since the sugar content in sugar beets has been so greatly increased, an increase of the protein of clover from 15 per cent of the dry matter to 16.5 per cent or even to 20 per cent should not be impracticable. But, important as may be the increase in nitrogen, breeding so as to adapt these crops to conditions where they now partially or wholly fail, and increasing their general yield and other good qualities in localities where they are now used, are the more important problems and probably should receive the first attention.

There is no reason why the nitrogen content of a variety can not be increased as well as the sugar content, the flavor, the hardiness, the height, or any other measurable characteristic. The Kansas experiment station found that ears of corn of a variety grown for thirty years on the same farm varied in protein content from 9 to 13 per cent, and that different varieties of corn varied about the same. Professor Hopkins, of the Illinois station, proved that corn plants with grain high in percentage of nitrogen generally produced grain with more nitrogen, thus proving that this quality can be improved, and he showed that something can be done at making the selections by mere inspection and without chemical analyses, though chemical analysis is a great aid. Those kernels which showed the largest proportion of dark reddish to white starchy interior, when cut across, as with a knife, had the highest percentage of nitrogen, thus enabling the careful farmer to select for more nitrogen.

ILLUSTRATIONS OF WORK IN PLANT BREEDING.

No attempt can be made here to go into the minute details of breeding the many species of economic plants. The discussion of wheat, corn, timothy, potatoes, apples, black walnuts, and flax in future pages serves to illustrate many of the general methods. Those who wish to engage in the breeding of any plant should first study that plant, paying special attention to its floral organs and to its method of pollination, to the methods of propagating and cultivating it, to the conditions under which it is to be grown, and to the purposes for which it is or might be used.

The literature will not be found extensive nor explicit in most lines, but by applying to his State experiment station, to the national Department of Agriculture, and to persons who have bred the plant concerning which knowledge is desired, the plant breeder can secure valuable information. The discussion which follows, besides showing present plans and results, is meant to be suggestive in relation to plans for breeding many other crops for which the experimenter must develop methods.

BREEDING WHEAT.

The breeding of wheat should not be confined to the few very best wheats, but a fairly large number of varieties may be profitably used, especially in forming new varieties by hybridizing. The best of these, if not all, should be placed under systematic field tests to determine their relative value, and to establish standards with which to compare newly originated varieties.

IMPROVEMENT BY SELECTION ALONE.

In nearly all cases wheat flowers are fertilized with their own pollen, and, in order that the occasional plants which have hereditary power of special value may be secured, it is necessary to plant large numbers of seeds in such a manner that each plant will have an opportunity equal to that given each other plant. At the Minnesota experiment station this is accomplished in the following manner: Twenty-five hundred good kernels of spring wheat are chosen from the bulk wheat of a good variety, either old or newly formed. These seeds are planted in hills 4 inches apart each way (5 inches for winter wheat), one seed in a hill. A dibble may be used to make the holes for the seeds, and the grains may be inserted and covered by hand. To get the hills the proper distance apart each way a large frame is used (see Pl. II, fig. 2). The long boards at the sides of the plot, or series of plots, have nails driven 4 inches apart on their inner edges, and the movable board has marks 4 inches apart across its front edge. When one row is planted the board is moved forward 4 inches to the next pair of nails. The series when planted appears as in figure 13. The dark circles represent plants which are to be removed immediately before harvest, as those shown on the border adjacent to the alleys have a larger food supply than those in the interior of the plot, and must therefore be removed before choosing the best ripe plants. Every individual plant has its number, and whatever notes are made concerning it are entered under that number. Alleys a foot or more in width are left between the series of plots that attendants may have a place to walk in planting, weeding, and harvesting the plants.

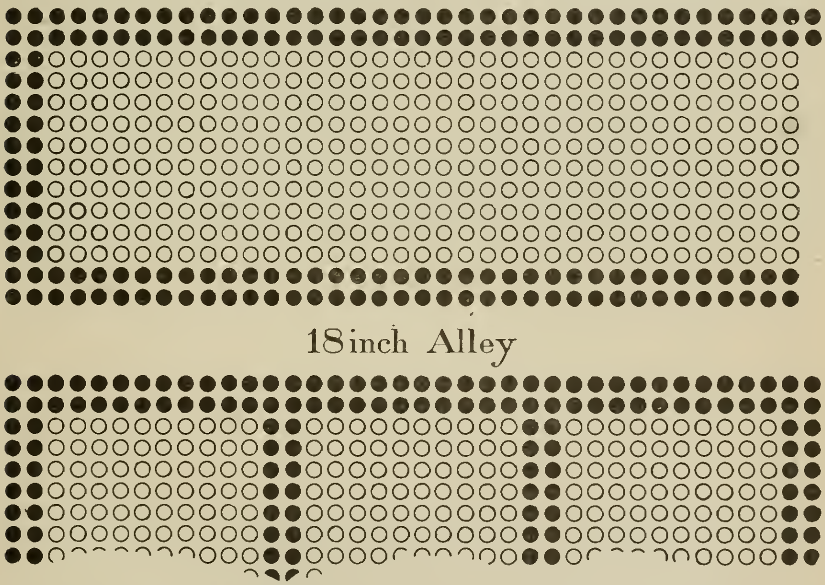


FIG. 13.—Scheme of planting wheat in field crop nursery. Light circles represent plants of the variety under experimentation. Dark circles represent another kind of wheat separating the plots from the alleys, or from each other.

Where a small number of plants is placed in a plot, this is separated from the adjacent plot of the series by a different variety of wheat, as shown by the rows of dark circles crossing the lower series in figure 13. Great care is used in preparing the plot to have the soil uniform, and to so arrange the ditches in the alleys that surface water will not run over the plots. The weeds are kept carefully removed by hand, and early in the season the soil is kept loose by stirring it with a very small hand hoe.

A new machine has recently been devised by the Minnesota experiment station for doing this planting. One to three seeds are planted in each hill, and when a few inches high all the plants but one per hill are destroyed. It is used for other crops also.

Harvesting and selecting the nursery-grown wheat is almost entirely a matter of elimination. A careful man removes from the plot about 95 per cent of the plants, leaving the comparatively few strongest and most desirable plants. In Plate III, figure 1, are shown two plots which contained 100 plants each From the one on the right 90 have been removed, leaving the 10 best standing. The spikes are cut from each plant separately and preserved in a packet or envelope. Where desired, the straw from each plant also may be harvested separately and placed in an envelope by itself; or, if thoroughly dry, it can be weighed at once. Where the variety has too weak straw, causing the crop to lodge, or where the straw is of little value as compared with the grain, the height, the weight, and the ability of the straw of each plant to stand erect are desired, that plants may be chosen which will increase the proportion of grain to straw.

When dry the contents of the packets are weighed. All the plants which are low in weight are at once discarded, even before shelling, reducing the labor of shelling to the few best. The shelled grain having been weighed to get the net weight, the grade or quality determined, and any other notes of interest made, the breeder is in position to make choice of the best plants. One hundred, more or less, of the seeds from each of these few chosen plants are planted in separate nursery plots in the wheat-breeding nursery the second season in a manner similar to that under which the seed was grown the first season. These collections of plants are called "centgeners," this word having been originated to mean a hundred plants, more or less, springing from the seeds of a single mother plant—that is, a large number of one generation. Each group of plants from a single mother plant thus planted in a centgener plat is given a nursery-stock number, written thus: "Nursery stock No. 17, 1892," this serving as a name for this stock so long as it is in the nursery. In case any stock becomes especially prominent in the wheat nursery, all or part of its seed is transferred to the field trials, where it is given a Minnesota number, as "Minnesota, No. 163, wheat." The nursery stock number, the class name of the parent wheat, also its Minnesota number, and any facts regarding its history, are entered upon a blank card, called "Introductory Sheet," which is placed as the first card in the history of the nursery wheats. The form of this sheet is as follows:

SELECTED STOCK—INTRODUCTORY SHEET.

(Minn. Form 61.)

WHEAT: Class name of parent stock, ——. Nursery stock No., ——. Minn. No. of parent stock, ——.

Date, ——, ——.

Origin and history of parent stock:

Size, 5½ by 8½ inches; color of card, white.

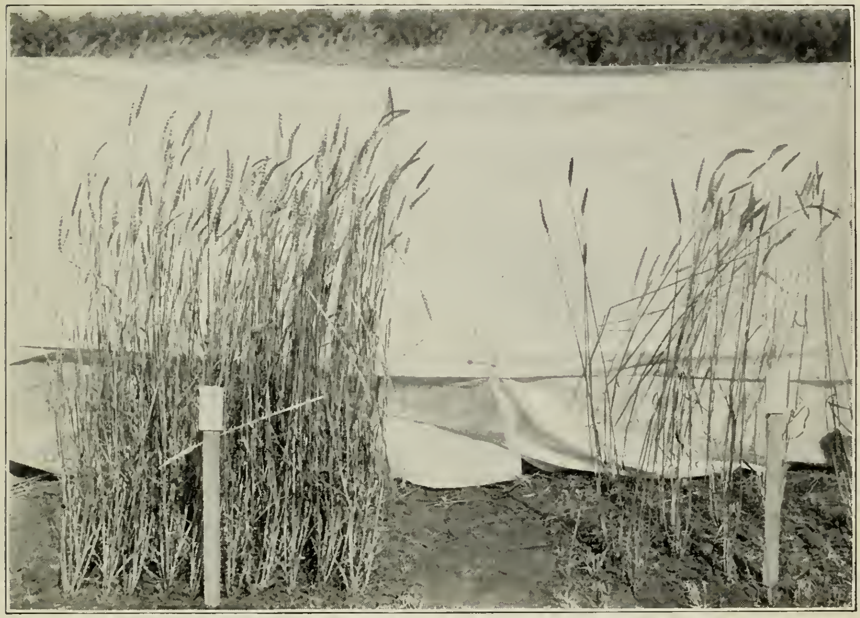


FIG. 1.—TWO CENTGENER PLOTS OF WHEAT.

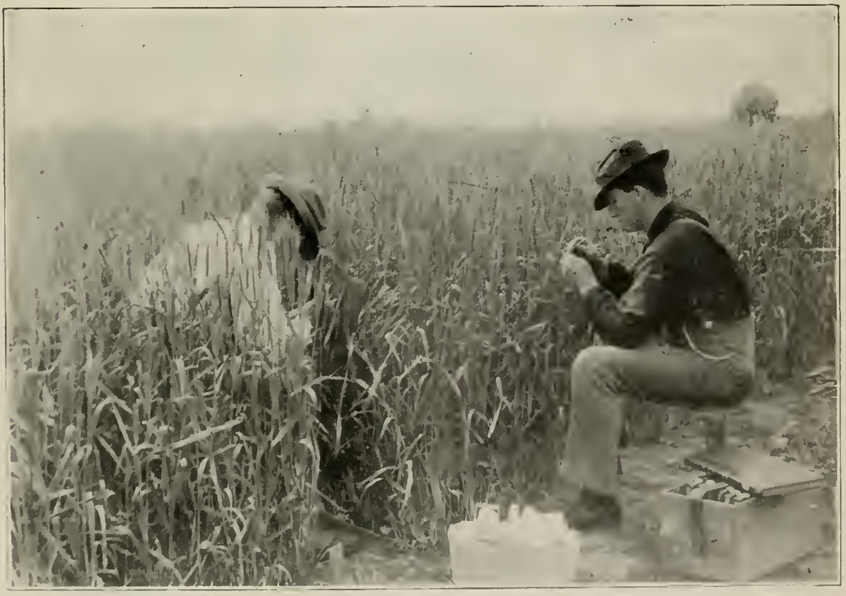


FIG. 2.—MEN EMASCULATING AND CROSS-POLLINATING WHEAT.

When the plants are ripe, notes are taken as to the relative size and strength of each plot. Each plant in the plot is pulled, counted, and placed in a bundle, and a choice spike is taken from each of several of the best plants that the best seed may be available to plant a centgener of this stock the next year. With a newly devised thrashing machine and a specially arranged fanning mill (Plate IV, fig. 2), which save every kernel from these small plots, the bundle is thrashed, cleaned, weighed, graded, and may then be analyzed for nitrogen. To the weight of this bulk grain is added that of the grain saved in the packet for planting the third year. This total weight is divided by the number of plants actually harvested, thus securing the average weight per plant of the progeny. The third and fourth years, a centgener is similarly planted, harvested, and tested. The records are kept on blanks in the following form:

YEARLY HISTORY SHEET.

(Minn. Form 63.)

Wheat: Class name ——. Nursery stock No. ——. From plant No. ——. Date ——. Cent. No. ——. Date ——.

CENTGENER NOTES.

Days maturing.	No. seeds planted.	Type.	Height.	Strength.	Stiffness.	Rust resistance.	Smut.	No. plants harvested.	Av. yield.		Nitrogen.	Note.
									Straw.	Grain.		

NOTES ON SELECTED PLANTS.

Nursery No.	Days maturing.	Height.	Stiffness.	Rust resistance.	Spikes.			Chaff.				Berry.					Yield of straw.	Yield of grain.
					No.	Length.	Missing.	Color.	Smooth or hairy.	Bearded or awnless.	Holds %.	Color.	Size.	Plumpness.	Condition of bran.	Grade.		

Size of card, 5½ by 8½ inches; color of card, white.

At the end of three years the data recorded in the yearly history sheets are collected and averaged for each stock, using the following form:

SUMMARY SHEET—CENTGENER NOTES.

(Minn. Form 65.)

WHEAT: Nursery stock No. ——.

Year.	Date planted.	No. seeds planted.	Type.	Height.	Strength.	Stiffness.	Rust resistance.	Smut.	No. plants harvested.	Av. yield.		Nitrogen.	Note.
										Straw.	Grain.		

Size of card, 5½ by 8½ inches; color of card, pink.

Then the averages for the various stocks—the progeny of the single mother plants, respectively—are compared by collecting them on such a blank as the following:

GRAND SUMMARY—CENTGENER NOTES.

(Minn. Form 67.)

WHEAT.

Nursery stock No.	Date planted.	No. seeds planted.	Type.	Height.	Strength.	Stiffness.	Rust resistance.	Smut.	No. plants harvested.	Av. yield.		Nitrogen.	Note.
										Straw.	Grain.		

Size of card, 5½ by 8½ inches; color of card, blue.

Thus the breeding ability—centgener power—of the various mother plants is tested and compared as to yield, grade (upon inspection), and percentage of nitrogenous content of the grain, and as to ability of the plant to stand erect, resist rust, etc.

The records of hybrid nursery stocks are in like manner kept on these cards. On the uppermost card are placed the facts concerning the two parent varieties and the objects sought in the cross. On the second card may be recorded the facts about each individual plant produced the second year by cross-pollinating a certain wheat spike. After having been grown for a few years to allow of free variation the hybrid stocks are dealt with year by year much as in the case of selected

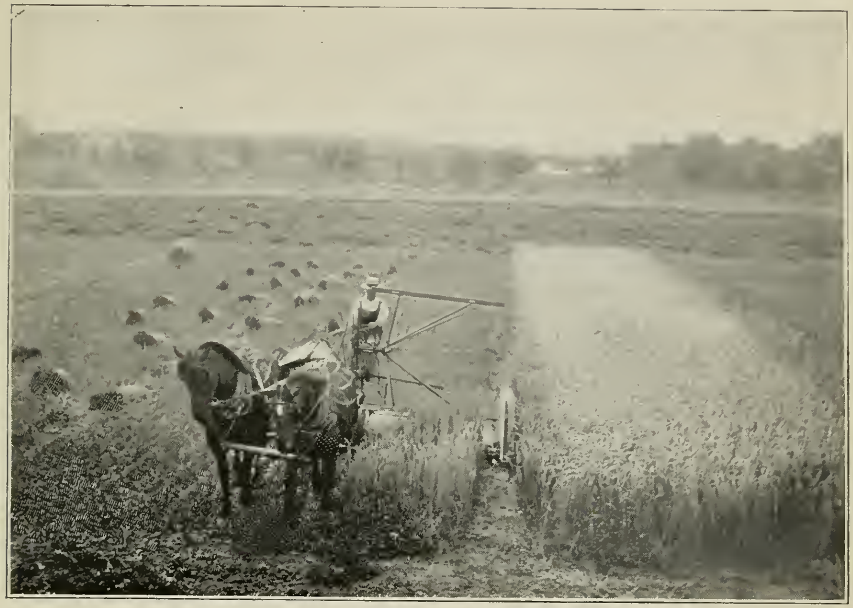


FIG. 1.—HARVESTING TWENTIETH-ACRE PLOTS OF WHEAT.

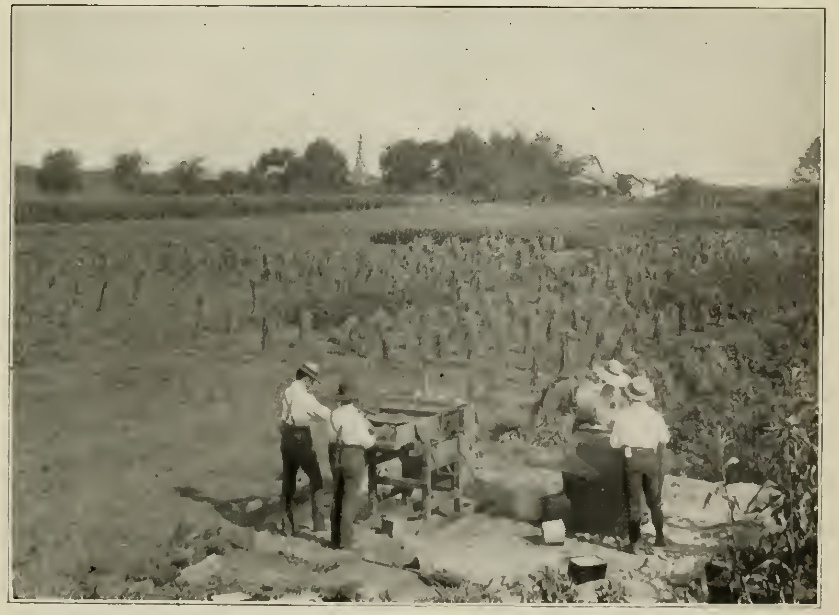


FIG. 2.—THRASHING AND CLEANING WHEAT FROM CENTGENER PLOTS.

stocks, and the same cards for the yearly records and the summaries are used as described above (see fig. 14).

At the end of the fourth year, when three annual centgener trials are averaged, all but the best stocks are eliminated. The seeds of those which have distinguished themselves are increased during the fifth year, and they are planted in uniform field-test plots in the sixth, seventh, and eighth years. The yields per acre, the grades, etc., are now averaged, and those which here distinguish themselves for yield, grade, etc., are given duplicated milling, baking (Pls. I and V), and chemical tests to thoroughly determine their real value to the miller and to the consumer as well as to the farmer. Some which prove second best, as well as those which prove worthy of immediate dissemination to the farmers, are sent to the substations within the State and to experiment stations in adjoining States in payment for similar favors from them, since it has been found that a wheat which is best in one locality is often not best in another locality. Once a new wheat has won its place as having yielded more value per acre at any experiment farm than the commonly grown varieties, its quantity is rapidly increased, and it is distributed to the farmers of the State or district, at current prices for seed wheat. Each farmer who receives this wheat is given a certificate of its genuineness as being the stock which made certain yields in comparison with the other wheats on trial at the experiment station. The farmer is requested to make reports of the yield and other qualities as compared with the wheat he is commonly growing on his farm, and he is expected to sell his first crops at remunerative prices to his neighbors for seed. In this way the final and most crucial test is given to a new strain or variety of wheat in each county in the State. The amount sent to each farmer is limited to a few bushels, and an effort is made to distribute each new kind to several farmers in each county in which it is likely to do

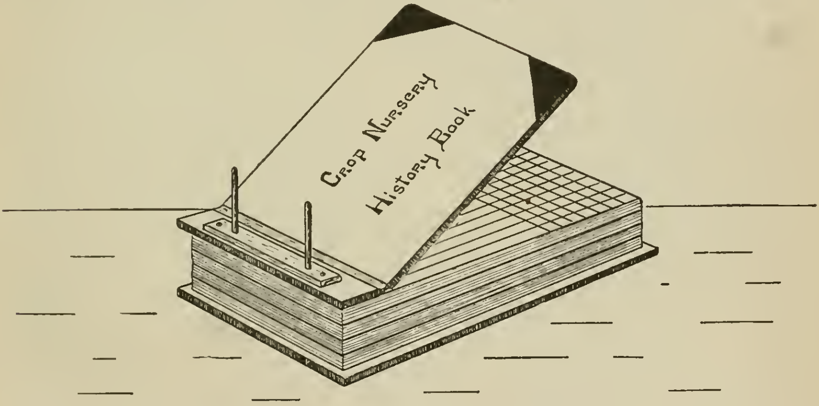


Fig. 14.—Method of preserving record sheets.

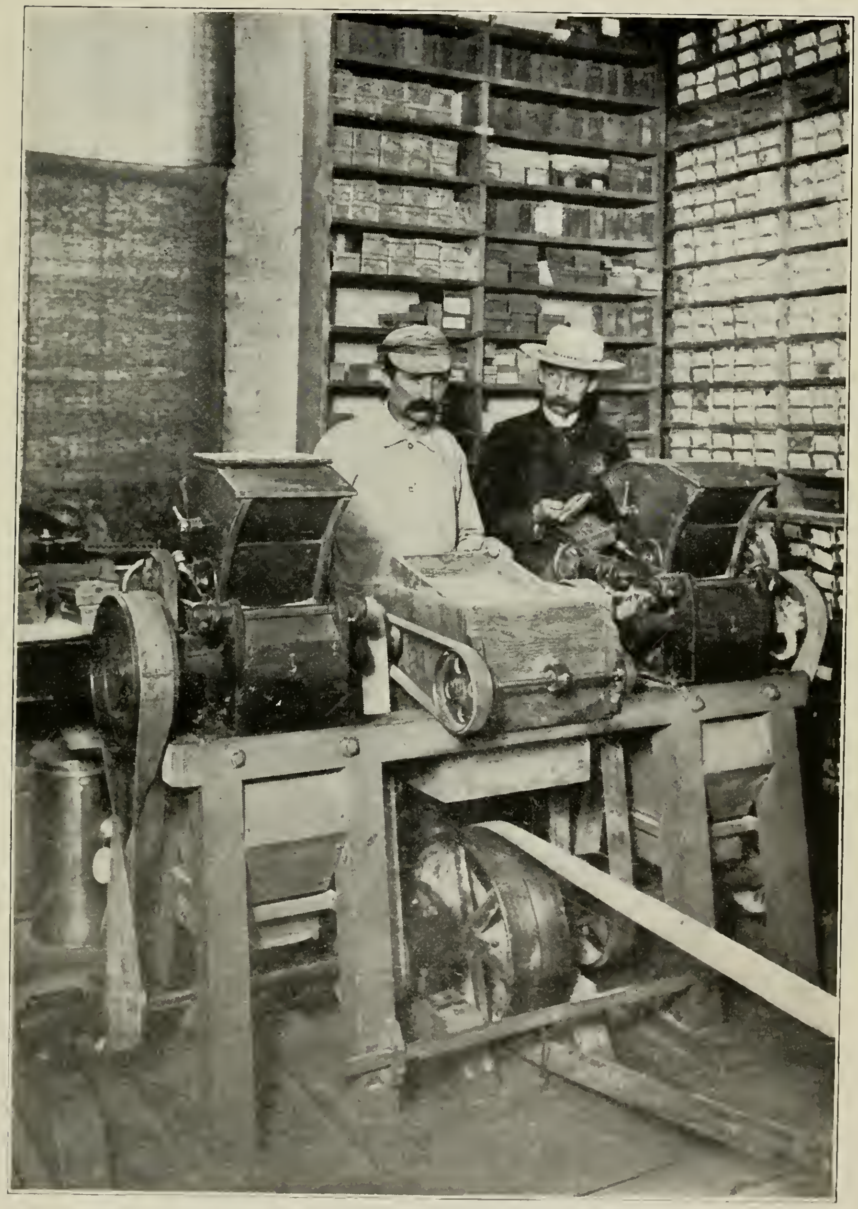
well. Without dissemination under some such system as the above, experiment stations would hardly be justified in breeding many of the staple crops, since only through successful methods of distribution do the farmers of the State receive any benefit.

WHEAT FLOWERS.

The floret is the most interesting part of the wheat plant. Figure 15 shows the separate parts of the flower, also the spike and the seeds. The floral plan is shown by the cross section at *5*, where the flowering glume (*fg*) and the palea (*p*) are folded about the three anthers (*a*) and the stigma (*s*). Before the flower is mature the anthers are closely packed about the stigma in the bottom of the floral cavity, as shown in *4A*. At the maturity of the flowers the anthers are shoved upward, some of them passing out of the floret, as at *4B*. The floret usually opens about dawn, and closes again within an hour. This is shown in figure 16, where the opening of the anthers is also illustrated, as shown in *4B* (fig. 15). In passing upward the pollen sacs break open, and before the anthers reach the outside of the floret some pollen falls back on the stigma. As the floret matures the stigma changes from its folded form, as shown at *12* (fig. 15), and expands into a plume (*13*). The pollen grain is a minute round male cell (*11*, fig. 15), which, falling upon one of the minute branches of the stigma (*13*), "germinates" and sends a tube into its tissues (*18*). This pollen tube, growing downward, enters the ovary (*13 o*), where its nucleus fuses with the female nucleus in the ovum, and from this fusion the embryo of a new plant arises. The stigma, having served its purpose, withers, while the ovary begins developing (*14*, *s* and *o*), and in a few weeks a mature seed fills the floral cavity. The seed has a ventral and a dorsal side, as shown in *15*, *16*, and *17*. At the bottom on the dorsal side is the germ, sometimes called "chit," the miniature plant, which is ready when planted to use the remaining portions of the kernel, the endosperm, as food while it sends leaves into the air and roots into the soil, establishing itself so that it can grow into a useful plant, multiplying itself many fold.

FORMATION OF VARIETIES BY HYBRIDIZING.

Hybridizing is used to produce plants with greater tendency to variation. Hybrids are made between numerous varieties of wheat, and in each case large numbers of florets are handled. Great care is exercised to secure superior plants of the varieties hybridized, and as a rule plants are chosen from the best centgener stocks which are under improvement by selection from the most useful standard parent wheats, as mentioned above. In preparing a good spike of wheat for hybridizing, all but one or two dozen strong florets in the center of the spike are removed by means of sharp scissors, as shown in figure 17. (See also Pl. III, fig. 2.) The anthers are removed from these



Milling Samples of Wheat.

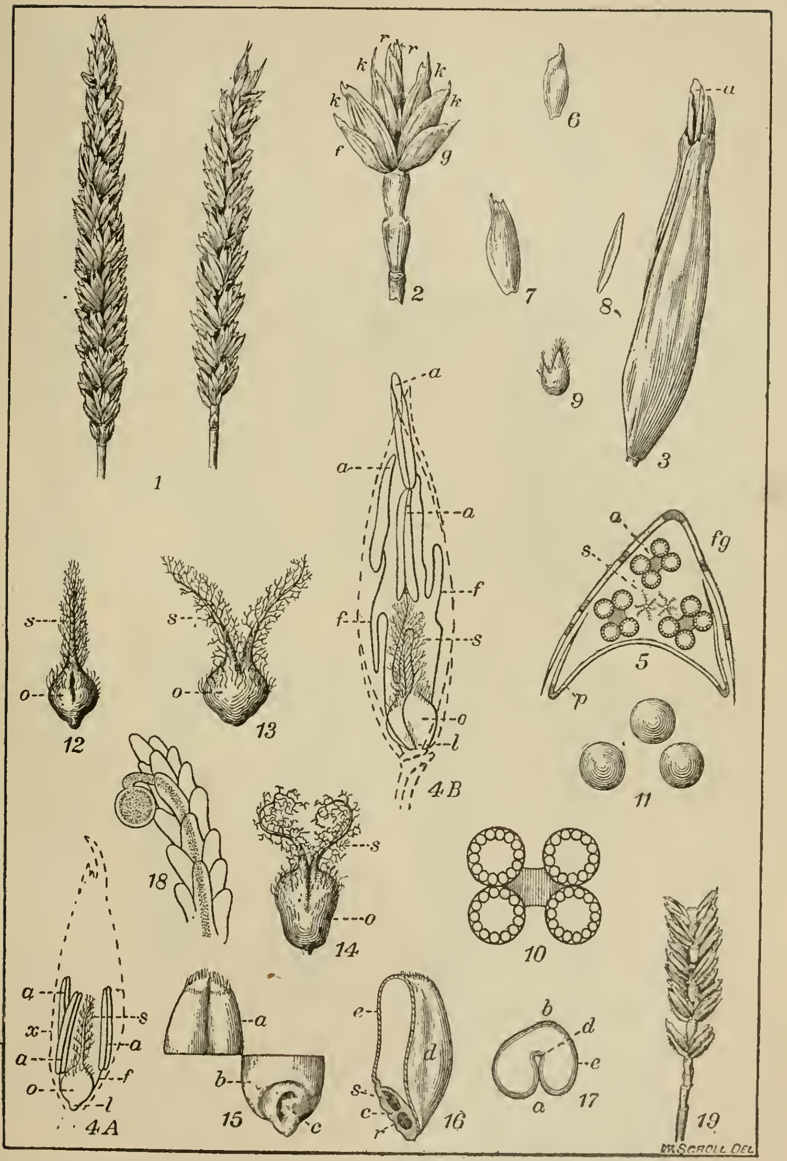


FIG. 15.—The spikes, flowers, and seed of wheat. *1*, spike of Fife at the right, and a blue stem spike at the left. *2*, spikelet (natural size), with a few joints of the rachis: *f* and *g* are flowerless glumes; *k*, florets bearing seeds; *r*, rudimentary florets. *3*, a single flower closed, just after flowering (×5). *4A*, longitudinal diagram before flowering; anthers marked *a*; ovary, *o*; stigma, *s*; filament, *f*. *4B*, diagram of floret just after flowering, showing how anthers are held within envelope, lettered as in *4A* (×5). *5*, transverse diagrammatic section or floral plan, as is made by cutting across *4A* at X; *fg*, flowering glume; *p*, palea; *a*, anthers; *s*, stigma. *6*, flowerless glume (natural size). *7*, flowering glume (natural size). *8*, palea (natural size). *9*, lodicule (×4); shown also at *l* in *4B*. *10*, cross section of anther, showing the pollen sacs and the central mass of tissue to which they are attached (×30). *11*, pollen grains, round and smooth, 55 micro-millimeters in diameter. *12*, ovary and stigma just prior to flowering. *13*, at the time of flowering. *14*, shortly after flowering. *15*, *16*, and *17*, the mature seed; *a*, the ventral side; *b*, the dorsal side; *c*, the germ, or chit; *s*, the stem end of the germ; *r*, the root end of the germ; *e*, outer layers of the grain, or bran; *d*, the incurved surface of bran on the ventral side of the seed. The white portions of *16* and *17* are the floury interior, consisting of cells containing the gluten and starch from which white flour is made. *18*, portion of the stigma, showing an attached pollen grain which is germinating and sending its tube down to the ovule. *19*, spike from which small late flowers have been removed preparatory to crossing.

remaining florets, as shown in figure 18, this being done when the flower is yet young and the anthers green or only slightly tinged with yellow. The emasculated spike is covered by wrapping about it a piece of tissue paper, tying above and below to prevent the accidental introduction of foreign pollen. One or two days later, when the flowers are fully developed, as shown by the opening of flowers of the same age on neighboring spikes, pollen is brought from the variety chosen for the male parent and inserted into the emasculated florets. The cross-pollinated spike is again covered with the paper, to keep out other pollen. The several resulting grains from each handled spike are stored in a packet and so planted the second year that the plant resulting from each seed has its individual plant number in the nursery. The entire product of each plant is harvested, and full

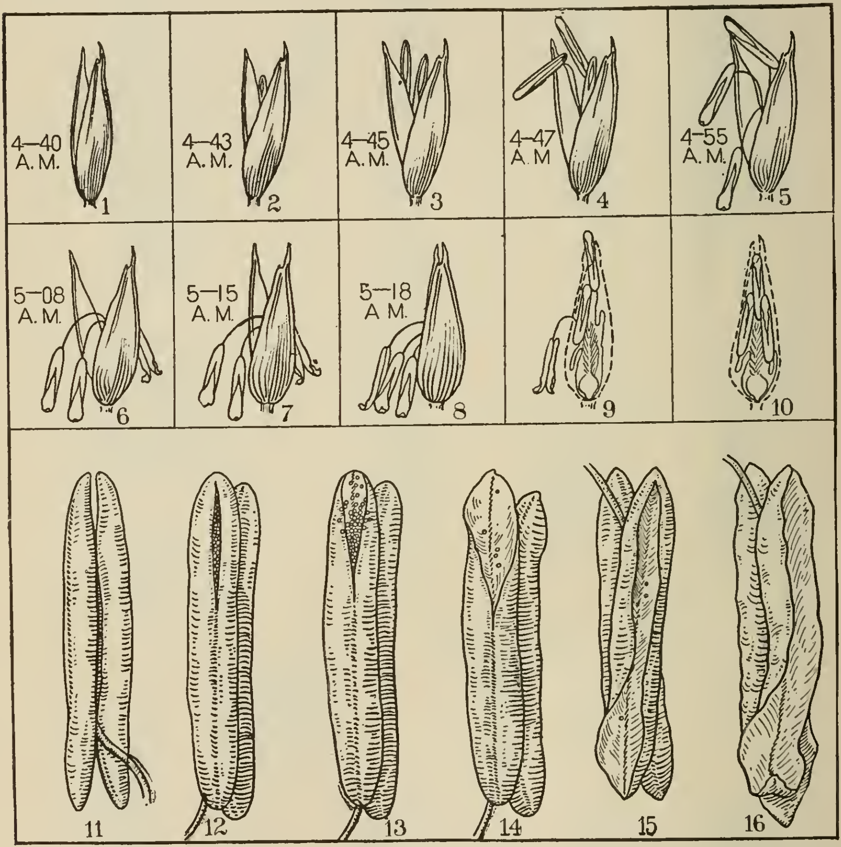


FIG. 16.—Opening of wheat flowers and anthers. *1* to *5*, opening of a wheat flower; *6* to *8*, closing of same; *9*, closed flowers, with one anther hanging out; *10*, wheat flower entirely closed; *11*, the anther, with its attachment to the filament; *12* to *16*, the progressive changes in the opening of the pollen sacs; *15*, *16*, anthers which have fallen out from the flower, thus inverting their position and allowing the remaining pollen to fall out, the anther becoming shriveled and brown.

notes are taken on the plant and on the seed. One of three general methods may now be used:

Method No. 1.—Each hybrid seed is separately planted the second year. The third year a centgener is planted from each original hybrid plant of the second year, and from among the plants of this centgener are chosen superior plants for mothers of centgeners the fourth year. The fifth year, variation having largely spent its force, centgeners can be grown and tested as under the plans given on pages 44 to 49, inclusive.

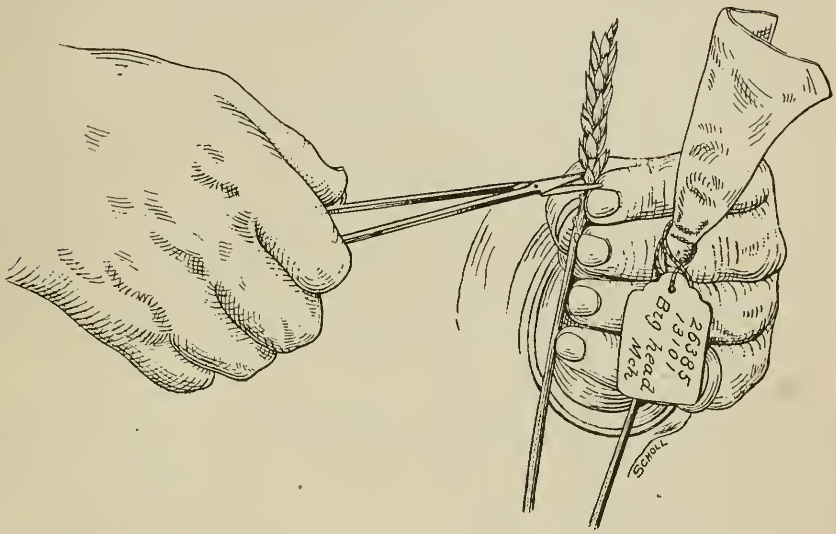


FIG. 17.—Removing the flowers of the smaller later spikelets at the end and at the upper base of the spike, leaving only the strongest florets to be emasculated and supplied with pollen from another plant. In front of the operator's left hand is a spike which has been cross pollinated, then covered by wrapping about it a piece of tissue paper, which is tied on.

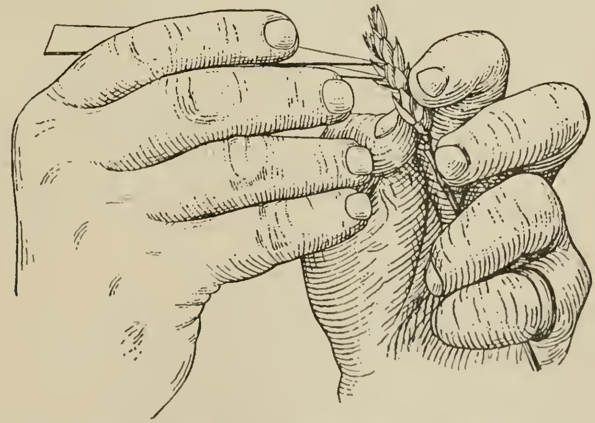


FIG. 18.—Opening the florets to remove the anthers.

Method No. 2.—Each hybrid seed is separately planted the second year. A nursery centgener plot is planted the third year from the seeds of each strong plant of the second year, and several of the best yielding plants of each promising type are saved for mothers and mixed together, to be used as nursery stocks, planted in a large nursery plot the fourth year, and treated in centgener and field trials as described on pages 44 to 49.

Method No. 3.—All the seeds of a hybrid between two stocks, or plants, or spikes, as the case may be, are planted in a nursery plot the second year, and the resulting crop of seeds is mixed together and planted in drills or broadcast the third year, and again the fourth year. By this time the "reactions" have mostly developed. The fifth year 2,500 hills, 4 by 4 inches apart, are planted, and thinned to one plant in a hill. These plants are then used the same as foundation stocks of standard wheats, as stated on page 44, the hybridizing having given wider variation, increasing the opportunity to select superior mother plants.

Method No. 3 seems the most simple and the most comprehensive. It may be wise in many cases to mix together two or more closely related nursery stocks for use in the field tests and later used by farmers, avoiding any possible danger of founding a variety upon the blood of a single mother plant.

EXPERIMENTS IN WHEAT BREEDING.

The actual formation of superior varieties, which at an early date will "make two blades of grass grow where one grew before," is of immense value, but of immeasurable importance would be the fullest knowledge of how best to breed into each and every species of useful plants and of animals those qualities which make them more useful in supporting and giving pleasure to humanity. Enumerating some of the lines of experimenting on the theory of wheat breeding in progress at the Minnesota experiment station may suggest similar studies by other experimenters with the various plants upon which the respective States depend for a large proportion of our country's wealth. The numbers at the left refer only to the station records. The statements in parentheses under the respective experiments are brief statements of results to date:

IV. 2. Seeds compared from heavy *vs.* light-yielding spikes.
(Results to date show that the breeder should choose the heavy-yielding spikes as well as heavy-yielding plants.)

IV. 3. How to select wheat plants for greater ability to stand erect.
(The centgener plan of experimenting is aiding in the solution of this important problem. The tendency in the blood of a mother plant to beget a race with stiff straw can not well be judged with the single plant, but it can with the small plot of a hundred or more of the progeny.)

IV. 4. Methods of developing earliness.
(By hybridizing and using centgener plot selection.)

IV. 6. Making better varieties by improving each of two stocks by selection *vs.* first hybridizing the two varieties, and then selecting.

(Recent results from incrosses and outcrosses lead to the belief that hybridizing is of paramount importance to supply the best stocks for the more laborious work of selection.)

IV. 8. Time required to reduce hybrid types to good yield, then to uniformity of type.

(Hybrid wheats vary as to the length of time variation continues under rigid nursery selection, but generally they are reduced to a type in a few generations, this being accomplished with little special effort while selecting for superior yield and other qualities.)

IV. 10. Study of best methods of planting field-crop nursery.

(Important modifications of the plan of breeding annually result from these experiments.)

IV. 12. Comparison of yield of crop from large-yielding plants with crop from poor-yielding plants.

(The selection of large-yielding individual wheat plants is important as a means of securing strong plants to be tested in centgener trials to determine their power of producing plants with large average yield.)

IV. 15. Does crossing and hybridizing increase variation?

(Many results show this very plainly in numerous characteristics, both those of botanical interest and of economic value, as yield, percentage of protein, etc.)

IV. 17. Method of breeding for strong chaff, which prevents easy shelling.

(By means of hybridizing and centgener-plot selection, good results are promised.)

IV. 22. Breeding wheats for special soils.

(Extensive trials of new and old wheats show conclusively that wheats must be especially bred for each of several conditions in Minnesota, as well as for each of the several adjoining States.)

IV. 23. Effect of changing seed wheat from one locality to another.

(The change in the new crop of seed is marked; sometimes for the better, often for the worse. General facts for practice are not yet available.)

IV. 27. Methods of seeking the best plants in the centgener or in the large nursery plot of stock seed.

(By inspection, the choice of 5 per cent of the best-appearing plants nearly always includes the plant which gives the largest weight and superior grade of grain.)

IV. 28. Does environment modify the individual, and are acquired characters transmitted?

IV. 29. How much do wheats cross in nature?

(Natural crosses do occasionally occur; percentage of such has not yet been determined, but it is very small.)

IV. 30. Methods to use in breeding for rust resistance.

(Records on the rust resistance of 100 progeny of each of numerous parent plants, made in percentages, promise to aid materially in finding blood lines which resist rust within the standard variety and among the plants of the new hybrid.)

IV. 35. Vigor of parents *vs.* the vigor of progeny of different degrees of relationship.

IV. 36. Should plants adjoining blank hills be thrown out in nursery selection and in scientific nursery studies of variation, etc.?

(One or two blank hills have so little effect on the yield of adjoining plants, where the hills are 4 by 4 inches apart, that in selection to form new varieties no heed need be taken of them. In the study of scientific questions, however, two or three grains should be planted in each hill, and early thinned to one plant, that the stand may be complete, giving to all plants an equal chance.)

IV. 37. Making a strain or variety of wheat from a single mother plant *vs.* from several mother plants.

(The comparison is not completed. But numerous strains, each from a single mother plant, have been grown for eight years in field-test plots, and they continue to average as much superior to the parent variety as at first, showing, so far, no signs of deterioration.)

IV. 38. Distance apart for wheat plants in the field-crop nursery.

(Four inches apart each way for spring varieties and 5 inches apart for winter varieties have proved the most satisfactory.)

IV. 39. Methods of handling the spike in cross-pollinating wheat.

(The best of several methods tried is as follows: Remove the smaller upper and lower spikelets and the smaller florets on the central spikelets, leaving ten to twenty of the best. Emasculate these early, about the time the first tinge of yellow appears in the anthers. Cover the spike with tissue paper. Twenty-four to forty-eight hours later, when the florets on neighboring spikes of similar age are opening, bring pollen from the plant chosen for the male parent and, removing the covering, apply pollen to each floret.)

IV. 42. Methods for treatment of hybrids during the first several years.

(One conclusion is that wheat hybrids should be grown in quantity during the first three to five years, that variation may have its full opportunity; then the selection of superior plants should be from among large numbers, as from among several thousand in the nursery plots.)

IV. 49. Crop from mother plants with low percentage of nitrogen *vs.* crop from mother plants with high percentage of nitrogen.

IV. 69. Should the plants in the wheat nursery be fed heavily, medium, or lightly in seeking plants best adapted to heavy yield in the ordinary field?

HYBRIDIZING AS A CAUSE OF VARIATION IN WHEAT.

In Plate VI (fig. 1) are shown spikes of two parent wheats, and between them an average spike of their hybrid progeny, as selected in 1895 by Mr. Warren W. Pendergast from the hybrid wheats at the Minnesota experiment station. In the upper row the right-hand spike is the Blue Stem parent, the left-hand one the Fife parent, and the central spike is the average spike of the single hybrid plant of the first generation. The spikes in the middle and lower rows are forms which appeared in the 100 plants of the second generation, all of which came from seeds from the single plant of the previous year. The "reaction" here was unusually strong, and the types of wheat produced are neither like the two parent plants nor yet intermediate between them, but several are very much like various of the so-called "species" of wheat. Henry Vilmorin, of France, showed the writer most of the so-called "botanical" classes of wheat growing in his garden, all of which were produced by hybridizing two varieties. He believed that this is proof "that all the domesticated wheats originated from a single species." It certainly indicates blood relationships between the classes of wheats. Whether this is wholly the result of

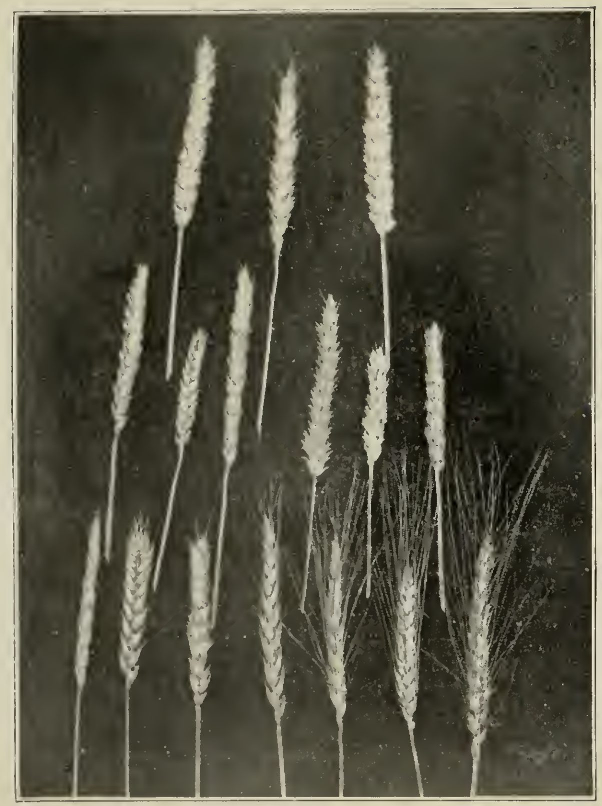


Fig. 1.—Spikes of Parent and Hybrid Wheats.

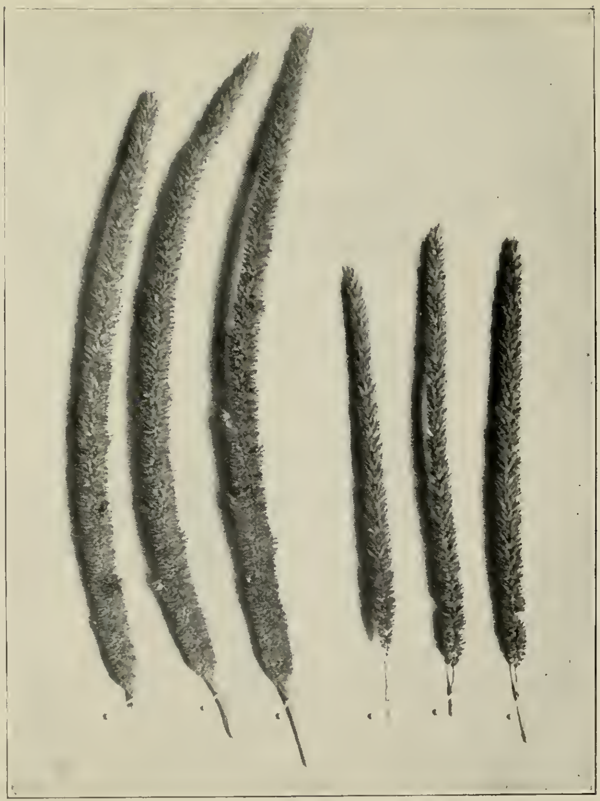


Fig. 2.—Spikes of Timothy, showing Improvement by Selection.

the differentiation of a single original species into subspecies, races, and varieties, or whether it is in part due to hybridizing between original species, may be questioned. It is also a remarkable illustration of the intricate relationships existing in nature even among plants apparently exclusively self-fertilized.

In figures 2 to 7, inclusive, with accompanying text and notes, the fact of the creation of variation in yield of wheat plants by hybridizing is also illustrated.

In 1893, from a floret of Blue Stem wheat pollinated from a Fife plant there resulted a seed which in 1894 developed into a plant, No. 1814 in figure 19. In 1895 a centgener of plants was grown from the 1894 mother plant. Of these, 30 per cent had smooth chaff, resembling the Fife parent, and 70 per cent had hairy, velvety chaff, resembling the Blue Stem parent. In the succeeding years smooth-chaffed plants were chosen for mother plants from one stock selected for the development of a smooth-chaffed variety, and plants with velvety chaff were chosen as mother plants from another stock selected for the development of a hairy-chaffed variety.

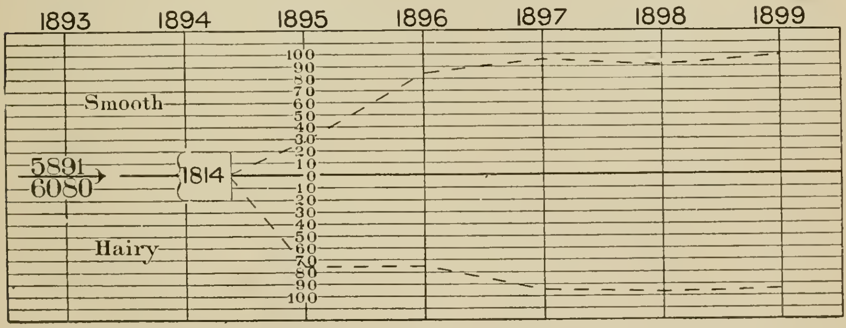


FIG. 19.—Graphic expression of the results of an experiment in developing from a single hybrid plant, No. 1814 (produced by crossing a plant of Fife with one of Blue Stem) two varieties, one having smooth and the other hairy chaff.

In figure 19 it is shown graphically that in the third generation each was nearly true to type, and that it remained practically true to type. Other hybrids under experiment are not all reduced to type so rapidly, as plants vary greatly in their tendency to continue departing from type; but if hybrid plants can thus easily be reduced to type in regard to largeness of yield, content of nitrogen, etc., and afterwards or simultaneously be in like manner easily made uniform in appearance, the improvement of wheat by cross breeding will not appear very difficult.

BREEDING CORN.

Corn breeding illustrates some of the principles of practice applicable to species which by nature are open-pollinated. Since the male and female flowers are on separate portions of the plant, the female florets are more often fertilized by pollen from neighboring plants than by pollen from the tassel of the same plant, and the individual plants of corn, being of mixed blood, vary greatly. Corn is easily improved, selection and hybridizing being easily and very effectively applied in making new varieties which will suit definite conditions. The careful selection of seed in the field by our farmers is changing corn for the better very rapidly. Experiment stations, seed firms, or farmers who wish to enter upon more careful corn breeding, so as to get better and more rapid results than usual, will find the following plans helpful.

SELECTING SEED CORN.

The first selection of corn is made from the field containing the varieties chosen for foundation stock. Since the plant is the unit in breeding, ears are chosen from each of several hundred of the best plants, and the shelled corn yielded by the plant is weighed. Where practicable, nitrogen determinations of the grain from each plant may be made, so as to eliminate in this first selection all those mother plants which are low in their percentage content of protein compounds; or the percentage of nitrogen may be roughly determined by choosing ears in which the grains appear glutinous rather than starchy when cut across.

Seeds from 100, more or less, of the best of these plants should be chosen, and the second year a centgener plot should be planted from each. The centgeners are planted in single rows placed side by side, 100 hills or more in each. The rows are planted 3½ feet apart, with the hills a foot or more apart in the row. Two seeds are planted in each hill, and when the corn is several inches high it is thinned to one plant in the hill, thus providing each plant with the same room as each other plant. At this point one of several plans may be followed.

Plan No. 1.—When mature, all plants of each centgener are harvested and dried, the grain is shelled out, and the grain, cobs, and stalks are separately weighed. These weights are divided by the number of plants actually harvested, to get the average yield of the plants from each mother plant. Notes are made of the characteristics of the plants, as height of ear, height of plant, etc., and an analysis of the mixed grain from all plants gives the yield of nitrogen per plant of each stock; or inspection of the kernels, cut across, showing the proportion of dark nitrogenous to white starchy substances, gives a fair index of the content of protein. Only part of the seed from each mother plant having been required for the centgener tests the previous year, there is an abundance of seeds of each of those mother plants which produced superior centgeners for again planting the third year. By planting the corn nursery the third year to centgeners from these best plants the poorer blood is discarded and the blood of the best mother plants is [illegible]. To here further discard undesirable blood, the weaker plants are detasseled just before flowering, thus preventing their pollen falling upon the silks of choice stalks. Several of the best-appearing plants are now chosen from each centgener, and tested as to weight and quality of grain, etc., that the very best may be secured for mothers of centgeners the fourth year. In following years the same plan is pursued. Seed of any stock which has distinguished itself in the nursery can at any time be taken to the field, multiplied, tested beside standard varieties, and, if it there proves superior in yield and quality, distributed to the farmers. Varieties which become prominent in the field may be again introduced into the nursery and subjected to rigid breeding; and while in the field, careful field selection should also be carried on.

Plan No. 2.—A somewhat simpler method is to select superior plants from among the centgeners as grown the first year under Plan No. 1 and at once continue the nursery selection as there described for the succeeding years.

Plan No. 3.—A still simpler plan is to plant and test centengers as in Plan No. 1 and save sufficient seed from the best plants in the best centgeners to plant a field the second year. Careful field selection of seed could then be carried on for one or more years and the plan of first-year centgener selection, as in Plan No. 1, could be repeated by again selecting seed from the field.

Plan No. 4.—Careful field selection may be made effective, as it has the important advantage of very large numbers to select from. When husking the corn from the standing stalks, as is the practice in Iowa and surrounding States, choose ears from many superior plants. By weighing, and by inspecting or analyzing for quality of gluten, eliminate all but the best. In many cases where hand husking has given way to the husking and shredding machines, results in yield of grain per acre may be more rapidly reached by breeding for two-eared dent varieties. Where corn is shocked, to be hand or machine-husked later, the seed should be saved before the corn is cut, while the entire stalk can be observed. In order to get the ears from the best plants it is necessary to husk—or at least to strip back the husks from—five times as many large ears as are to be saved for seed. Where two or more fields are planted to the same variety the choicest seed should be planted in one field and seed chosen from there for the next year's planting.

While it may be practicable to use only one mother plant as the basis of a variety in a close-pollenized species like wheat, this should not be done in a species like corn, which is accustomed to free mixture of parental blood. McClure, in Illinois, found that self-fertilizing in corn caused a loss of vigor, and suspected that self-pollination in the cornfield may be responsible for many of the barren stalks so commonly found in our cornfields.

HYBRIDIZING VARIETIES OF CORN.

In the work of variety formation hybridizing is very useful. Before hybridizing two varieties of corn it is best to carefully select each so as to get mother plants of superior worth. Any one of several simple plans may be pursued in cross-pollinating.

First plan.—Alternate rows of each may be planted and one kind detasseled, so as to insure pollination by the other. Seeds from the resulting ears may be planted for two or three years, that the mixing of blood may continue in the production of variations. From a field of at least an acre of this mixed corn superior plants may then be chosen for planting, as indicated under the plans of selection outlined above.

Second plan.—Seeds of the choice plants of two varieties may be mixed in bulk and planted in the corn nursery, one seed in a hill, as above mentioned, so that mixing may occur naturally. By detasseling or removing all but the best plants before flowering time, only good plants will cross, and the best plants may be chosen for mother plants.

Third plan.—Hybrids may be made by hand pollinating, (a) choosing superior plants in two separate stocks in carefully planted nurseries; (b) choosing plants in a nursery where the two varieties are planted in alternate rows; (c) choosing plants in two ordinary fields, either near together or far removed from each other.

In hand pollinating, cloth bags 10 by 20 inches are used to cover the ear of the plant of one variety and the tassel of the other variety to be crossed. These are slipped on and tied a few days before the flowers mature. The bag on the ear is removed—temporarily protecting the ear from other pollen by an umbrella—and pollen from the bag containing the tassel is dusted upon it. This should be repeated daily for two or more days that the ear may be fully fertilized. The further breeding of the mixed stocks from the hybrid ears thus produced may be done in a variety of ways, but in any case large numbers of plants should be grown for two or three years, that the variations inaugurated by the mixture of the two races of blood may have ample opportunity to go forward. These stocks may be planted in the field for a few years, where a very large number of plants may be used, and from which in the second or third year superior mother plants may be chosen, that their seeds may be taken into the corn nursery for further selection, as already outlined under "Selecting seed corn."

Since the nitrogen determination can now be so cheaply made, it would seem that the breeding of corn with heavier yield and with a higher percentage of nitrogen could be made profitable on an extensive scale. Nitrogen added to our corn crop would be as valuable as sugar to the sugar beet. The addition of nitrogen will no doubt be much slower and more difficult than the addition of sugar, but should amply repay the State or private plant breeders for the expense. Careful notes, records, and pedigree summaries can be worked out for the mother plants and their centgeners of progeny in careful breeding of corn in much the same manner as has been outlined in the breeding of wheat. The yield, the per cent of nitrogen, the size, form, and character of the stalk are individual characters which should be numerically recorded in securing superior parent plants; and in testing these mother plants by comparing large families of their progeny the centgener yield, percentage of nitrogen, etc., should be recorded, averaged, and compared.

Farmers can and do materially improve many of their varieties of corn by crossing and selecting, and in some cases they injure good varieties by injudicious crossing. Once a superior variety is obtained, it should not be allowed to be mixed with another or replaced by another without the best of proof that the change will be for the better.

BREEDING TIMOTHY.

In 1889 the writer gathered seeds from numerous timothy plants found along the wayside and on farms in the neighborhood of the Minnesota experiment station. A hundred or so seeds from each mother plant were planted in a plot, one seed in a hill, the hills being 12 by 18 inches apart. When these plants were 2 years old, each having stooled, making a bunch a foot or more across, the best plants were chosen from the best plots. Seeds from these best plants were harvested and plots were similarly planted, and this process was repeated for succeeding generations. About 50 plants of the third generation were divided up into settings, which were transplanted into plots a square yard in area. When these were 2 years old, seeds were saved from the best plots, and this seed was sown to increase the stock of seed from these varieties. The variations among the plants was sufficient to warrant us in attempting to select some of the stocks as mothers for the development of meadow varieties and others for pasture varieties. The seed has now increased to sufficient quantity for making field tests of the yield of dry matter and the yield of nitrogen per acre in meadow or pasture plots.

It so happened that some of the very best plants had a tendency to long spikelets, and several of the 14 stocks which are being increased in stock-seed plots have barbed spikes, such as are shown in Plate VI, fig. 2. The three spikes on the right represent the foundation stock from which was developed by selection the new timothy represented by the three spikes on the left, which shows a tendency to branch by lengthening some of its spikelets. A distinguishing mark like this would have value in a new kind of timothy, since it would distinguish it from common timothy, which has not as yet been broken up into successful varieties. But in the end the historical method by numbers used for names may be the most practical way of keeping track of the strains and varieties of timothy, because a variety of barbed timothy could easily be broken up into strains or subvarieties, some of which would have greater value than others. Breeders are prone to breed for the distinguishing marks and to exert their energies in making the new botanical characteristics come true to type, rather than to seek first the yield per acre and quality of the grass and hay. The difficulty of planting these minute seeds in hills in the field and the gain of a year in the maturity of the plants has led to the plan of planting the seeds in greenhouse pots in winter and transplanting to the field in the spring. Since timothy is an open-pollinated species, it would seem unwise to base efforts for its improvement upon a single mother plant. Effort should be directed to finding superior individuals and mixing together their seeds for breeding purposes. To

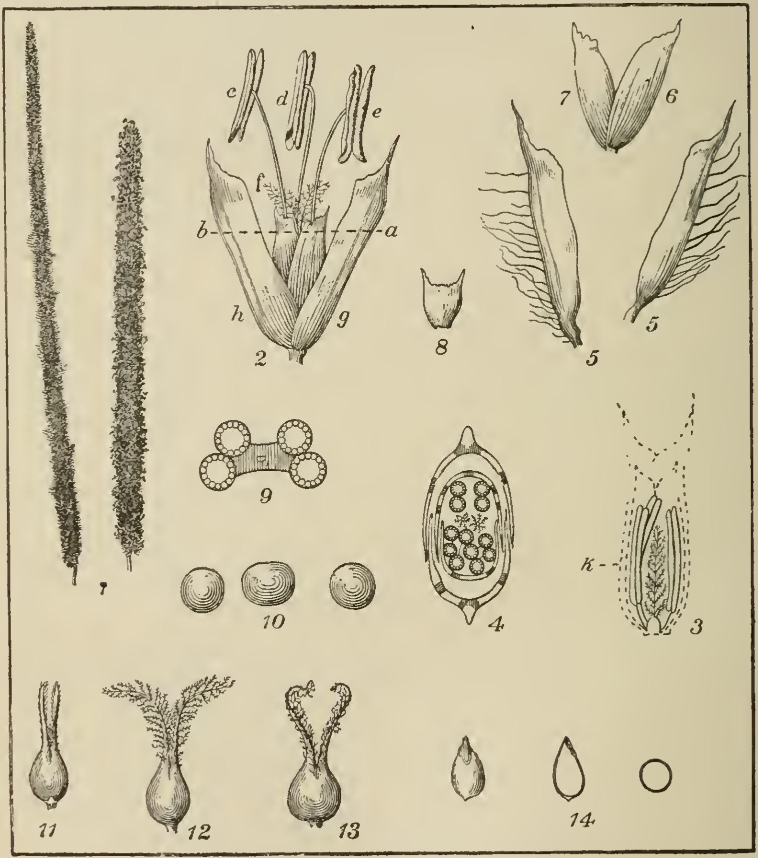


FIG. 20.—Floral organs of timothy. *1*, two types of spikes, the righthand one in bloom, the lefthand one a new variety, showing extended spikes. *2*, a single flower: *h*, *g*, empty glumes; *a*, *b*, flowering glumes; *c*, *d*, *e*, anthers; *f*, stigma. *3*, longitudinal diagram of flowers showing position of organs within the unopened glumes at *k*. *4*, transverse diagram of flower. *5*, outer or empty glumes. *6*, flowering glume. *7*, palet. *8*, lodicule. *9*, transverse diagram of anther. *10*, pollen grains. *11*, *12*, *13*. pistil before, at the time of, and after pollination. *14*, outer, longitudinal, and transverse appearance of seed.

increase variation, timothy seeds from widely separated sources may be mixed together and seeds from the mixed planting planted in the nursery. Or the stocks of seeds from separate sources may be grown in the nursery and the seeds of the best selected plants may then be mixed together and allowed to cross, thus giving crosses between strong parent plants. In any case these cross-bred stocks may be selected as under the plans given in the first part of this section.

BREEDING POTATOES.

Potatoes will serve as an example of a species the improved varieties of which are propagated wholly by cuttings, the seeds being used only in variety formation. The tops live only one year. The age to which a variety propagated by annually planting the root cuttings of a single seminally produced plant will live before the necessity of renewal by sexual reproduction is resorted to is not known. But since standard varieties of potatoes remain prominent for only about a third of a century, there is some reason for the belief that the varieties reach their period of old age, or senility, in that time.

Some of the many commonly grown varieties produce many seeds, but others produce very few seeds; and under some conditions most of the cultivated varieties bear very few seed balls. Doubtless those best acquainted with the formation of varieties of potatoes may have a knowledge of which varieties, or hybrids between which varieties, have proven best to use in making new sorts. There are annually many new kinds created, but only a very small part of 1 per cent of these ever reach the prominence of commercial sorts. Luther Burbank is quoted as saying that not more than one new variety of potato in five thousand should be named and disseminated.

The potato flower is quite open, and cross-pollination by hand is easily effected. The fruit, "potato ball," contains numerous seeds, each of which is capable of being developed into a new variety of potato. New varieties may be originated by planting seeds which have not resulted from crossing between varieties; but the supposition is that a larger proportion of good kinds would result were hybrid seeds from hand-pollinated flowers used.

The seeds are planted in the open field or under glass, and when several inches high the plants may be transplanted into the field, where each plant should be in a hill by itself, each having its serial number. In radical hybrids very poor seeds, and even plants which at first appear undesirable, sometimes become the foundation of superior varieties. The plants do not generally grow large the first year, and the tubers are small. By discarding all the tubers of the poorer three-fourths of the plants, only the best are retained for planting the second year. Several hills of each should be grown under proper nursery-stock numbers, so as to get averages, and when the tubers are mature most of the stocks grown this second year should be discarded. These are again planted, and those averaging poorest for the three years are discarded. Any very promising sorts are now rapidly multiplied for variety field tests. These new varieties are yearly compared with the standard sorts as to yield of tubers, cooking quality, form, size, and general appearance of tubers; where wanted for the production of starch they are tested for the percentage content of starch in the ripe tubers. Several kinds of apparatus for the determination of starch in potatoes have been devised. Other characteristics—as earliness, size of vines, and adaptability to certain soils and local conditions—are also noted. There has been very much effective work done in the breeding of potatoes by Burbank, of California; Heine, of Saxony, Germany; Archibald Finley, of Scotland, and many others. The transformation and constant improvement of this once wild American species is quite astonishing, and forms another remarkable example of what can be done by intelligent effort in plant breeding.

BREEDING APPLES.

The apple will serve as an example of an open-fertilized perennial species the varieties of which do not come true to type from seed, but are propagated mainly by grafting the buds or cuttings on other hardy stocks. While plants of this class require a number of years to bring them to the age of fruiting, and still longer to test their hardiness, quality, and yield, there is the great advantage which comes from propagating from buds or cuttings. There is not the necessity of breeding them to uniformity of type, because the cuttings and grafts, being only a part of the single seminal plant, are all true to type. This is also an excellent example of a plant which has been broken up into very many useful varieties by the discovery of superior trees which have come up accidentally, as along fence rows or about cider mills.

There is considerable variation in plants from seeds of self-fertilized apples grown in orchards where the trees are not near trees of other varieties; but much more where the trees of different sorts are close together, resulting in cross-pollination by the wind and by insects, and where the seeds are from fruits of flowers which have been cross-pollinated by hand. Many of our good varieties of apples have sprung from seedling trees produced from seeds from self-pollinated flowers or from seeds resulting from natural hybridizing in orchards where the trees of two or more varieties are adjacent. But in systematic work in apple breeding it is believed that more is accomplished with a given expenditure by artificially crossing those better varieties which combine the desired qualities, and thus producing many variable plants, any one of which proving of value may be rapidly propagated by grafting or budding for dissemination as a new variety. There is an important advantage in the systematic method of cross-pollination.

Records can be kept of the crosses made, and, plants from each cross having been tested, the results can be compared, and thus those crosses which have given the largest proportion of useful varieties can be known, so as to use them more extensively in future hybridizing.

The individual seed and the tree springing from it being the unit of the entire variety, each individual apple plant should be given a number, and notes should be recorded for each tree from its birth to its rejection, or until its selection as the mother of a variety for dissemination.

Each variety used as the parent of a hybrid may be considered as one plant, since all came from a single seed. If from Wealthy-Oldenburg hybrids made in large numbers there resulted wonderful variation with many trees producing good apples, all of which mature in autumn, we would expect further breeding of this cross to produce mainly varieties which would not keep in winter. If hybridizing Rhode Island Greening-Oldenburg produced mostly winter keepers, even though only a small percentage of good ones resulted, we should look more to this combination to produce the long-wished-for hardy varieties of winter keepers which are needed to push the winter-apple zone northward. The characteristics of individual trees of the "fraternity" group designated in this article as the "centgener" should, so far as practicable, be recorded in numerical averages, that one cross may be compared with another. Not only will our experimenters be able thus to learn which varieties are best to cross, but the more careful work will result in a better knowledge of the best ways of breeding apples.

HOW TO HYBRIDIZE APPLES.

There is such a multiplicity of conditions for which varieties of apples are desired that the work of variety formation of apples is largely creative. The Minnesota Horticultural Society offers a premium of $1,000 to the originator of an apple as hardy and productive as the Oldenburg, equal to the Wealthy in size and flavor, and to the Malinda in keeping quality. A permanent committee, with Prof. S. B. Green, of St. Anthony Park, Minn., as chairman, has charge of the awards.

Prof. N. E. Hansen, of the South Dakota Agricultural College, says:

> The Northwest at present needs varieties of applies combining the hardiness and freedom from scab of the best Russian varieties with the choice quality and long-keeping capacity of our best American winter varieties. A variety as hardy and large as Hibernal, as choice in quality as Northern Spy, and as long a keeper as Ben Davis or Romanite would be worth millions of dollars to the prairie Northwest.

Professor Hansen is crossing and hybridizing these types and other American and Russian varieties, as Siberian and hybrid Siberian crabs, large-fruited native crabs, Chinese red-flesh crabs, seedless apples, and other forms of apples from various parts of the world. He urges that an abundance of trees of a new variety should be started, that the new variety may be given a thorough trial, not only for a few winters, but until "bearing and test winters occur together," so as to thoroughly test their hardiness, and he is testing many new means of aiding the trees to endure the winters, as dwarfing, potting, grafting on hardy species, etc.

Prof. S. A. Beach, of the New York experiment station, says: "The breeding of apples in an unsystematic way is going on all over the country. I know of a rocky pasture where seeds were distributed by cows which had access to the pomace of a cider mill, and now thousands of trees are in bearing there. We have grafted about 75 selected kinds from these trees. The varieties commonly grown have mostly originated in this way, and may be looked upon as the few survivals from thousands upon thousands which have been allowed to fruit in neglected places." He has made many crosses and grown from seeds of self-bred flowers for the great apple regions of New York varieties which are superior to those now in use. His effort in his systematic crossing is "to combine features which exist in varieties which are known, but which are not combined in the way we most desire." Pure seedlings are grown "of the same parents to discover what features they may be expected to impress most persistently and firmly upon their progeny, and thus produce parents which will be most apt to transmit their characteristics with certainty." If Professor Beach succeeds in his plans he will have varieties "with attractive red fruit, ripening here in midwinter or later, approaching Ben Davis in regular bearing, vigor, and health, and considerably excelling it in quality of fruit." Looking toward this end, he is growing seedlings from the following crosses: Ben Davis-Esopus (Spitzenburg); Ben Davis-McIntosh; Ben Davis-Gravestein; Ben Davis-Green Newton. In like manner other varieties are being crossed to produce fall and winter apples which have red fruit of excellent quality and other good characteristics, as health, productiveness, etc.

Prof. John Craig, formerly of the Iowa Agricultural College, who was breeding apples extensively, said:

> (1) In order to do the work of crossing expeditiously and in a large way, it is necessary to have a quantity of pollen at hand. This, of necessity, will have to be collected from a region south or west of the point at which the work is to be done, and where the apple blooms earlier. In collecting it the blossom clusters are either picked from the branches, or a branch of considerable size is brought into a greenhouse or warm room. This is done just as the flower buds begin to open. In twenty-four to forty-eight hours the anthers will have burst, and they may then be removed with the pollen. This is quickly done by clipping them off with scissors. If a considerable quantity of pollen of a given variety is collected, care should be taken not to bottle it up closely immediately after it is gathered. Heating is likely to ensue, and the pollen may be rendered valueless. It should

be spread on sheets and dried, not to absolute dryness, but so that it will remain in powderly form, rather than adhere in pellets.

(2) Good, vigorous trees are selected. Strong blossom clusters are chosen. The outside buds are rejected, and three to four of the strongest buds near the center of the cluster are selected. In the case of large apples only two buds are chosen in a cluster. The work of emasculating should begin when the buds have fully expanded, but have not yet begun to open. The stamens may be removed by the use of a small pair of sharp-pointed forceps; I prefer these to straight or curved scissors. Care should be taken in doing this work that pollen from branches above is not transferred to the stigma of the blossoms being operated upon. The emasculated blossoms are covered with brown paper sacks. In the Eastern States, where more atmospheric humidity is present, and where there is a larger percentage of cloudy weather, it is desirable that paper sacks of a light weight should be used. The sack is attached to the twig or branch by means of a string which is tied to one edge of the sack, and is used to draw the mouth of the sack tightly together about the branch. Each cluster is then labeled with an ordinary nursery tag attached to the twig with a copper wire.

(3) The work of pollinating may be very much expedited if a variety of pollen is inclosed in a small homeopathic vial, and with it a small camel's-hair brush. In working on a large scale I find that it is much more satisfactory to, in the first place, collect an abundance of pollen and use it freely than to gather the pollen as required and apply it with any other instrument than the camel's-hair brush. Having a vial labeled and supplied with its own brush, all parts of the work can be done by the same operator, namely, the work of removing sacks, applying the pollen, and replacing the sacks.

(4) The paper sacks, if tightly tied on (which is necessary in this locality on account of the strong winds), will prevent the full development of the fruit if allowed to remain on more than ten days or two weeks after pollination. They should then be exchanged for sacks made of mosquito bar. These are cheaply made, costing about 1 cent each. They are tied on by having a running string around the mouth, which is closely drawn about the branch. The sacks protect the fruit from bird attacks, and to some extent from insects; they also prevent it from being lost if blown off by high winds.

(5) The common copper wire nursery label is used. One label is placed on each sack, and first records the name of the female and then that of the pollen-producing parent, as, for instance, "Mercer-Ben Davis, '99." The records of the seedlings may be kept by prefixing a numeral in each case and using the initial letter of each parent, as "1 M.-B. D., '99;" "2 M.-B. D., '99," etc. Each individual tree from every crossed seed planted is recorded and numbered separately.

As a rule, I do not plant the seeds of apples which are not crossbred. Occasionally a considerable quantity of seed of a special variety is planted, and in this case the trees are not recorded until they come into bearing. Then only those giving special promise are numbered.

Prof. J. L. Budd, of the Iowa Agricultural College, has made many apple hybrids, using the Russian varieties freely, and many of these are now coming into fruitage. Several of Professor Budd's students, having gained inspiration from him, are now breeding apples and other plants on an extensive scale.

Mr. C. G. Patton, of Iowa, the originator of Patton's Greening and other good seedlings, has long appreciated the possibilities in apple breeding. Luther Burbank is said to have made progress in making varieties suited to California conditions. He grafts scions of the new seedlings on standard apples, sometimes over 500 new kinds on one large tree, that all may be tried under similar conditions. Prof. S. B. Green, of the University of Minnesota, is breeding apples. He says:

> We know little about which varieties to use for superior crosses. The matter is of such immense importance that we should make very many hybrids between very many varieties.

BREEDING BLACK WALNUTS.

The black walnut serves well to illustrate the breeding of those forest and ornamental trees which are propagated by seeds. Walnut trees are grown for large logs of valuable lumber. Therefore the planter needs varieties which will make rapid growth and will mature early into trees of large size, straight, and of good form. In selecting seeds to plant, growers usually get nuts wherever they are secured with greatest ease. This leads to taking most of the seeds from heavy seed-bearing trees rather than from those trees which make a rapid and large growth of lumber. In case of this tree the nuts have some value, but in many species the seeds are of no use except to use in propagating, and if the tree bears many seeds it must do so at the expense of the production of wood.

It might seem that the breeding of walnut and other trees is impracticable because of the long time required to get results. But the time is not so long as might be supposed, as will be brought out by the following suggested plan of securing superior varieties of walnuts.

Since the pollen-bearing organs and the ovaries are in separate flowers, the flowers are often cross-pollinated from other trees, and there is considerable variation and opportunity for selection among trees from nuts of the same mother tree. Likewise, there is great variation between the plants grown from the nuts from several trees growing native in one neighborhood, and doubtless still greater among plants from mother trees found native in widely separated portions of the country.

The writer, over twenty years ago, in central Iowa, planted some acres to black walnut, and, the method of planting proving very good and the distance apart about right, the suggestions here are in part based upon that experience. The nuts were from various large and small native trees along a neighboring stream. They were gathered when sufficiently ripe to be easily shaken to the earth; and were at once placed in trenches 6 inches deep and 2 feet wide, running down a slight incline in the shade of a grove. Moist straw or leaves were placed over the nuts, from which the hulls had not been removed. The nuts were thus kept moist all winter, that they might be cracked by freezing. In the spring the fall-plowed land was marked off each way with a corn marker, making cross marks nearly 4 feet apart each way. The nuts were planted in each hill of every third row, thus placing them 4 feet apart in rows 12 feet apart. Some nuts will lie in the ground one or two years before germinating, but by planting three in a hill and thinning to the one strongest plant a full stand can be secured. Two rows of corn or potatoes were grown between each two rows of trees for three or four years, thus giving a partial crop to in part repay the thorough culture and weed killing. Thus cultivated, the trees grew very rapidly, some bearing nuts in ten years. In the twelfth year the poorer plants were removed, yielding 125 posts per acre, worth $12.50, and sufficient fuel to pay for thinning and making the posts.

If a plan such as the above is adopted and the nuts from the various native trees are planted separately, by the twelfth year choice can be made between them as to their value as mother trees, and nuts from the largest well-formed trees can be chosen for further planting. The seeds of two or more of the best of these trees may be planted in alternate rows, so as to allow of natural hybridizing in the next generation.

Cross-pollinating may also be done by hand among the best specimens. Those between native stocks brought from widely separated regions would be most likely to vary, and thus give opportunity to select useful new forms.

But even this need not be the limit of breeding operations. Luther Burbank's cross between the Eastern black walnut (*Juglans nigra*) and the California walnut (*J. californica*) illustrates the fact that the species of our forest and nut-bearing trees will in some cases hybridize, and these radical crosses made in immense numbers, followed by rigid and extensive selections, are sure to result in the production of useful new hybrids quite as marked as ordinary species. The trees in the grove mentioned above are now about 40 feet high. They are proving a profitable investment, and breeding them as suggested above could have been done at slight additional expense.

BREEDING FLAX.

In flax we have an example of the breeding of a species yielding two distinct valuable products—seed and fiber. So far as the writer knows, there has been no attempt, except in the Minnesota experiment station, to systematically breed varieties of flax for seed and for fiber. For this State the common blue-flowered flax has been found best. This flax has been imported from Russia at various times during the past thirty or more years, and the presumption is that the flax generally grown in large quantities for seed in Minnesota and surrounding States is the variety which has been long in use in Russia. While White Dutch and other kinds of flax have been tested for raising crops of seed, the Russian variety retains the supremacy, and is best in this climate for fiber also.

The flax grown for fiber in the British Islands and on the western portion of the European continent is nearly all of Russian origin.

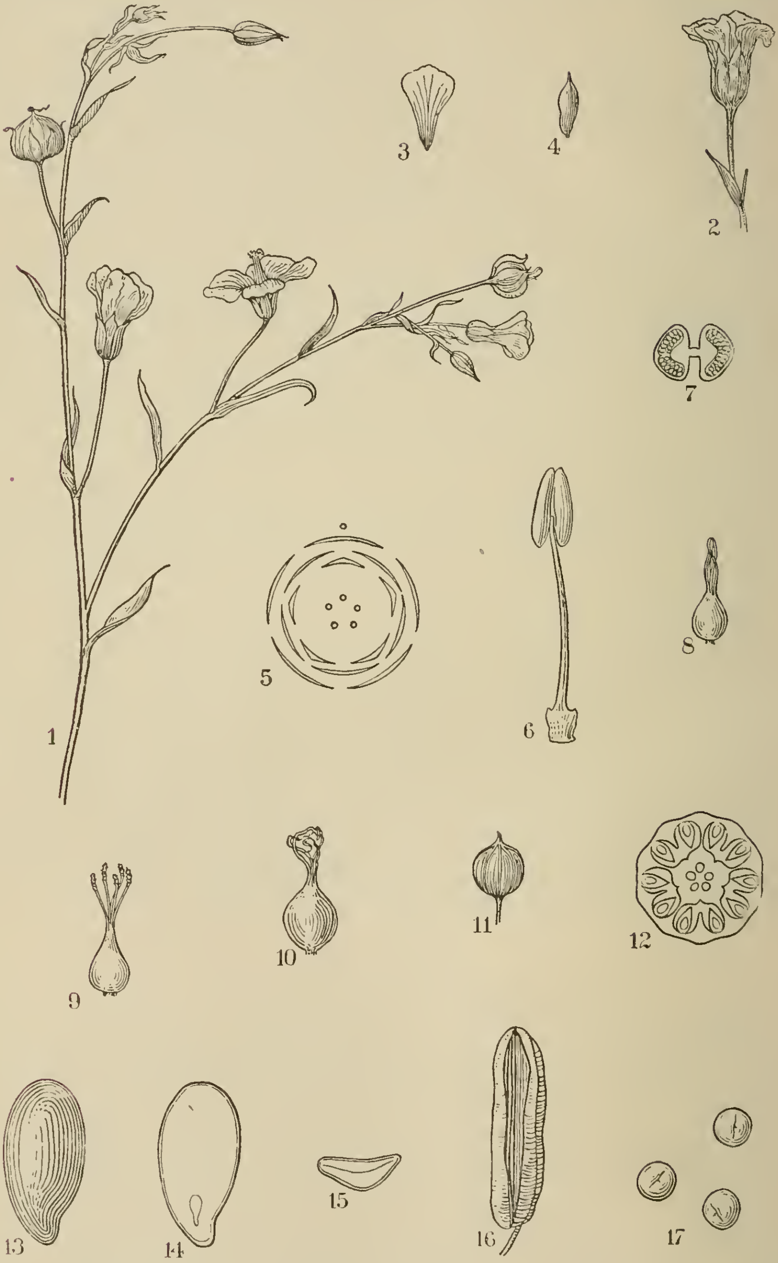


FIG. 21.—Flowers of flax. *1*, branch, with flowers and fruit; *2*, flower half open; *3*, petal; *4*, sepal; *5*, diagrammatic section of flower; *6*, anther, with filament; *7*, transverse section of anther; *8*, *9*, *10*, pistils, before, at time of, and just after pollination; *11*, ripe fruit; *12*, diagrammatic transverse section of fruit; *13*, seed; *14*, *15*, longitudinal and transverse sections of seed; *16*, anther; *17*, pollen grains.

The farmers of Ireland, Scotland, Holland, and Belgium import their seed from the vicinity of Riga, Russia, and after growing two crops of fiber they import fresh seed. Americans who grow flax for fine fiber have also imported part of their seed from Riga and part from Holland. There is apparently only one variety of this Russian seed generally recognized commercially. At the Minnesota experiment station, Minnesota or Dakota grown flax has been repeatedly grown beside that from the Russian seed recently imported. The two were apparently identical, whether sown thinly for crops of seed or thickly for crops of fine fiber. It is reported that in some Russian districts are to be found varieties better suited for growing fiber in dry climates, and an effort is being made to secure them.

For the States of the middle Northwest two kinds are wanted—one to sow at the rate of 3 pecks, or less, of seed per acre for large crops of seed; the other to sow at the rate of 2 bushels or more of seed per acre to grow large crops of long, fine fiber. If a number of such fiber varieties were developed here some of them might be found especially suited to growing flax fiber in Ireland, Holland, and Belgium, and in other countries which now purchase their flaxseed from other countries; arrangements have been made for testing some of the new varieties already formed in the European districts which import the flaxseed for their fiber crops.

The plan developed for breeding flax may be briefly stated, as follows:

1. Secure various stocks or varieties of flaxseed, and, having tested them so as to choose one or more of the best, sow broadcast at the rate of not over 1 bushel per acre in plots of at least one-tenth of an acre.

2. With great diligence seek among the plants growing in the field plot a number of plants which are strong, tall-growing, or medium tall fiber plants, and a like number of ordinary height which bear heavily of seed.

3. From each of these plant a centgener, placing three seeds in hills 5 or 6 inches apart each way, and when several inches high thinning to one plant in the hill.

4. When mature, take notes on each centgener on a blank form, with headings somewhat as follows: Centgener No.; Height; Strength; Average yield; Tendency to tiller; Tendency to branch; Evenness of ripening. Doubtless the content of oil and of nitrogen in the seed can be increased, and also the fineness and the quantity per acre of the fiber, together with the ability to stand erect, though these latter qualities, being somewhat antagonistic, are blended in one variety with difficulty.

5. Discard 90 to 95 per cent of the plants, and take full notes on the remaining plants on a blank form like the following:

FLAX.

Nursery No		181
Type		Fiber.
Date when ripe		Aug. 15
Height	inches	40
Number of tillers		1
Number of branches		5
Number of bolls		6
Evenness of ripening	per cent	90
Size of seeds	do	97
Grade of seed	do	95
Gross yield of seed	grams	22.5
Net yield of seed	do	16.4

6. From among each of the best centgeners of each type choose a few of the best plants for mothers of centgeners the second year, and continue year after year recording, compiling, and averaging the facts.

7. When any stock has shown a superior habit of growth, yield, and quality, multiply it rapidly and test it in the field, and, if a fiber kind, test it also in the factory or laboratory for yield and quality of fiber in comparison with standard varieties. Laboratory methods are being developed for testing the strength and fineness of the fiber and for determining its percentage of the crop, so as to determine the yield of fiber.

8. It may not be wise to limit the parentage of a new flax to a single mother plant, as this is an open-pollinated species, but the seeds from three or more centgeners may be mixed together in attempting to make a new variety.

9. Stocks once started in the nursery will serve as excellent parents to use in creating new qualities by hybridizing.

10. Hybrid stocks may be grown in bulk for a few years, that variation may fully develop, the selection then being carried on as under paragraphs 2 to 8, inclusive.

11. Two varieties may be hybridized by mixing the seed and sowing for two or three years in a plot or field, from which superior mother plants may then be chosen, as already described.

12. New hybrid varieties should not be distributed to growers until they have been tested several years in uniform test plots.

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